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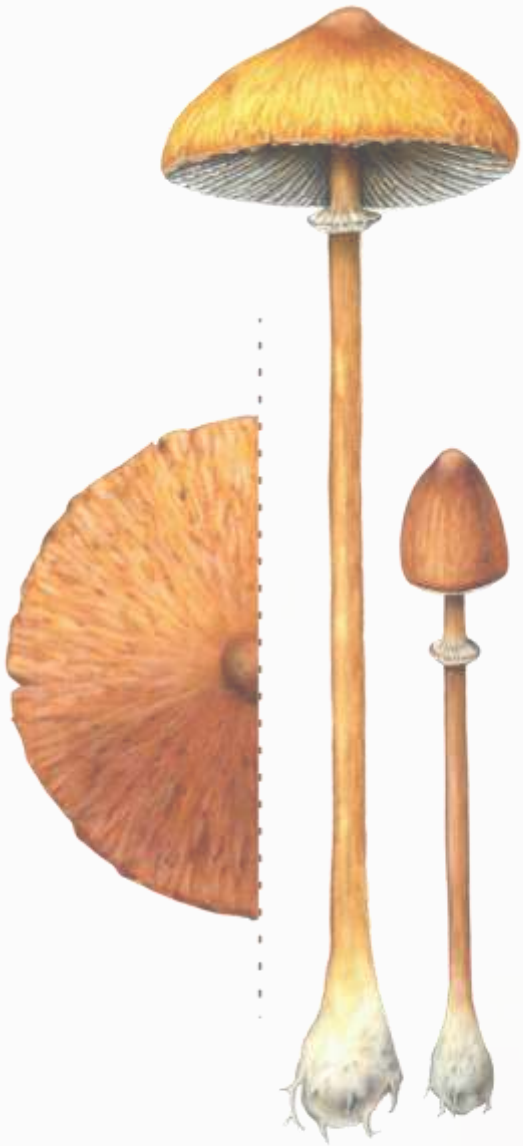
ORIENTADOR: DR. NELSON MENOLLI JUNIOR



Macrolepiota chapeletae C. Coelho-Nascimento & Menolli

Ilustração em aquarela: Dyego Costa

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À minha família,

meu micélio de apoio e feto, sempre presente sob a superfície. Com vocês, cresci em rede, forte e resiliente, mesmo nos solos mais áridos.

À profa. Dra. Maria Helena Alves,

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“Fungi are everywhere, yet we have only begun to understand their true diversity.”
D.L. Hawksworth

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Aos herbários FLOR, FIFUNGI, ICN, SP e UFRN - Fungos pelos empréstimos de coleções de grande importância nos estudos morfológicos realizados.

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A cada pessoa que, de algum modo, fez parte desta jornada, deixo aqui minha gratidão mais sincera.

Cristiano Coelho do Nascimento

RESUMO

No Brasil, o conhecimento sobre a diversidade e o uso de cogumelos comestíveis silvestres permaneceu, por décadas, negligenciado e fragmentado, marcado por identificações imprecisas e atribuição equivocada de nomes de espécies estrangeiras a táxons neotropicais. Nos últimos anos, contudo, a adoção de ferramentas moleculares e abordagens integrativas têm impulsionado avanços significativos no conhecimento desses macrofungos. Inserido nesse contexto, e diante do crescente interesse na conservação da funga e da demanda por fontes alimentares sustentáveis, este trabalho teve como objetivo investigar a diversidade, usos tradicionais a viabilidade de cultivo e os aspectos nutricionais de cogumelos comestíveis silvestres da Mata Atlântica brasileira, com ênfase nos gêneros *Macrocybe, Macrolepiota*, *Pseudohydnum* e *Tuber*. As coletas de espécimes fúngicos contemplaram principalmente fragmentos do referido bioma, com predominância nas regiões Sul e Sudeste, além de incursões pontuais nas regiões Centro-Oeste (Pantanal) e Nordeste (Mata Atlântica). Complementarmente, foram examinados espécimes depositados em herbários e fungários nacionais (FIFUNGI, FLOR, ICN, SP e UFRN-Fungos). Como resultados, para o gênero *Macrolepiota* foram descritas quatro novas espécies (*M. abruptibulbosa*, *M. capelariae*, *M. chapeletae*, e *M. pernudae*) e duas novas variedades (*M. capelariae* var. *velana* e *M. pulchella* var. *gymnopodia*), além da revisão morfológica e filogenética de três espécies previamente conhecidas (*M. bonaerensis*, *M. cyanolamellata* e *M. sabulosa*). Com exceção das duas últimas, a comestibilidade de todas as espécies mencionadas foi documentada pela primeira vez neste estudo, e três delas (*M. bonaerensis*, *M. capelariae*, and *M. chapeletae*) tiveram sua composição nutricional analisada, evidenciando teores expressivos de proteínas (30.65−35.48 %), fibras (14.34−15.86 %), potássio (1931.67−2003.33 mg/100 g), e fósforo (983.33−1383.33 mg/100 g). No gênero *Pseudohydnum*, foram descritas quatro novas espécies (*Pseudohydnum brasiliense, P. brunneovelutinum, P. cupulisnymphae* e *P. viridimontanum*), representando os primeiros registros moleculares e morfológicos completos desse grupo na região Neotropical. No gênero *Tuber*, foi descrita uma nova espécie (*T. bianchettiformis*) e documentado o registro inédito de duas espécies para o Hemisfério Sul (*T. lyonii* e *T. brennemanii*), além do primeiro registro de *T. maculatum* para o Brasil. Por fim, *Macrocybe titans*, um macrofungo comestível de grande porte nativo da região Neotropical, foi domesticado e cultivado com sucesso a partir de isolados silvestres coletados na Mata Atlântica brasileira. O crescimento micelial em meio BDA teve melhores resultados a 25 e 30 °C, sendo o sorgo mais eficiente que o trigo como substrato para produção de inóculo. O cultivo em substrato compostado contendo principalmente bagaço de cana e palha de braquiária resultou em uma eficiência biológica de

62.23% ± 0.10 para o isolado MPD643. Os basidiomas cultivados apresentaram alto valor nutricional, com destaque para os teores de carboidratos (52.9 ± 0.04 %), proteínas (1833 ± 0.20 %), potássio (31.033± 817.9 mg/kg), e fósforo (11.250 ± 736 mg/kg) . Em conclusão, os resultados desta pesquisa ampliam o conhecimento sobre a funga comestível neotropical ao trazer clareza taxonômica para importantes espécies de cogumelos comestíveis silvestres, registrar saberes tradicionais, além de demonstrar a viabilidade de cultivo de linhagens nativas de alto valor nutricional. Ao integrar diferentes abordagens, a tese oferece subsídios concretos para ações de conservação, segurança alimentar e fortalecimento da fungicultura no Brasil.

Palavras-Chave: Diversidade, *Macrolepiota*, Etnomicologia, *Macrocybe titans*, Domesticação

ABSTRACT

In Brazil, knowledge regarding the diversity and use of wild edible mushrooms has long remained neglected and fragmented, marked by imprecise identifications and the misapplication of names of foreign species to Neotropical taxa. In recent years, however, the adoption of molecular tools and integrative approaches has driven significant advances in the study of these macrofungi. Within this context, and in light of the growing interest in fungal conservation and the search for sustainable food sources, this study aimed to investigate the diversity, traditional knowledge, cultivation potential, and nutritional aspects of wild edible mushrooms from the Brazilian Atlantic Forest, with emphasis on the genera *Macrocybe*, *Macrolepiota*, *Pseudohydnum*, and *Tuber*. Field collections were conducted mainly in remnants of the Atlantic Forest biome, particularly in the southern and southeastern regions of Brazil, with additional surveys carried out in the Pantanal (Central-West) and in northeastern Atlantic Forest areas. In addition, specimens deposited in national herbaria and fungaria (FIFUNGI, FLOR, ICN, SP, and UFRN-Fungos) were examined. This study resulted in the description of four new species of *Macrolepiota* (*M. abruptibulbosa*, *M. capelariae*, *M. chapeletae*, and *M. pernudae*) and two new varieties (*M. capelariae* var. *velana* and *M. pulchella* var. *gymnopodia*), along with a morphological and phylogenetic revision of three previously known species (*M. bonaerensis*, *M. cyanolamellata*, and *M. sabulosa*). Except for the latter two, the edibility of all mentioned taxa is reported here for the first time. Nutritional analyses of *M. bonaerensis*, *M. capelariae*, and *M. chapeletae* revealed high contents of protein (30.65–35.48 %), fiber (14.34–15.86 %), potassium (1931.67–2003.33 mg/100 g), and phosphorus (983.33–1383.33 mg/100 g). In *Pseudohydnum*, four new species (*P. brasiliense*, *P. brunneovelutinum*, *P. cupulisnymphae*, and *P. viridimontanum*) were described, representing the first comprehensive morphological and molecular documentation of this genus in the Neotropics. In *Tuber*, one new species (*T. bianchettiformis*) was described, while two others (*T. lyonii* and *T. brennemanii*) are reported for the first time from the Southern Hemisphere, in addition to the first confirmed record of *T. maculatum* from Brazil. Finally, *Macrocybe titans*, a large edible macrofungus native to the Neotropics, was successfully domesticated and cultivated from wild isolates collected in the Atlantic Forest. Mycelial growth on PDA was optimal at 25–30 °C, with sorghum grains supporting faster colonization than wheat, indicating greater suitability for spawn production. Cultivation trials using a composted substrate composed mainly of sugarcane bagasse and *Brachiaria* straw resulted in a biological efficiency of 62.23 % ± 0.10 for isolate MPD643. The cultivated basidiomata exhibited high nutritional value, with notable levels of carbohydrates (52.9 ± 0.04 %), protein (18.33 ± 0.20 %), potassium (31,033 ± 817.9

mg/kg), and phosphorus (11,250 ± 736 mg/kg). In conclusion, this study advances current knowledge of Neotropical edible fungi by providing taxonomic clarification for key wild mushroom species, documenting traditional knowledge, and demonstrating the cultivation potential of native strains with high nutritional value. By integrating multiple approaches, this thesis offers concrete contributions to fungal conservation, food security, and the development of mushroom cultivation in Brazil.

Keywords: Diversity, *Macrolepiota*, Ethnomycology, *Macrocybe titans*, Domestication

LISTA DE SÍMBOLOS E UNIDADES

°: grau (ângulo plano)

': minuto (ângulo plano)

": minuto (ângulo plano)

°C: grau Celsius

ha: hectare

km^2: quilômetro quadrado

m: metro

mm: milímetro

µm: micrometro

s: segundo (tempo)

min: minuto (tempo)

h: horas (tempo)

gL^{-1}: grama por litro

Mt: Megatonelada

t: tonelada

kg: quilograma

g: grama

mg: miligrama

ng: nanograma

µL: microlitro

M: molar

mM: milimolar

µM: micromolar

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SUMÁRIO

Apresentação

Esta tese apresenta os resultados do projeto intitulado “Cogumelos comestíveis humícolas da Mata Atlântica: sistemática e estudos de domesticação”, desenvolvido no Laboratório de Ensino, Pesquisa e Extensão em Micologia (IFungiLab) do Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), *Campus* São Paulo. Este projeto foi desenvolvido, paralelamente, como um dos desdobramentos de um projeto mais amplo denominado “Cogumelos da Mata Atlântica: diversidade e potencialidades de espécies comestíveis”, financiado pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e iniciado em 2019 (Processo Fapesp #2018/15677-0) sob a coordenação do Prof. Dr. Nelson Menolli Jr.

O projeto principal de 2019 teve como objetivo central inventariar e caracterizar a diversidade de cogumelos da Mata Atlântica, com ênfase na identificação taxonômica, isolamento de linhagens, teste de cultivo e avaliação do potencial alimentar e comercial de espécies de cogumelos comestíveis silvestres. Um dos marcos iniciais foi a construção de uma listagem sistemática das espécies de cogumelos comestíveis silvestres do Brasil, elaborada a partir da revisão de literatura especializada, consulta a bases de dados internacionais e realização de novas coletas de campo acompanhadas de análises filogenéticas moleculares. Essa etapa revelou uma quantidade expressiva de registros controversos e inconclusivos, abrangendo espécies pertencentes a diversos gêneros, o que evidenciou a necessidade de investigações taxonômicas integrativas para validar a ocorrência e o uso dessas espécies no território nacional.

Nesse contexto, foi iniciado, em 2020, o projeto que fundamenta a presente tese, com o propósito de aprofundar o conhecimento sobre a diversidade e as potencialidades de cogumelos comestíveis da Mata Atlântica, especialmente aqueles associados ao solo. Ao longo de sua execução, o escopo do projeto foi ajustado, passando a incluir também espécies lignícolas, consolidando-se em torno do estudo aprofundado de quatro gêneros de reconhecido interesse alimentício: *Macrocybe*, *Macrolepiota*, *Pseudohydnum* e *Tuber*. Com isso, o título do projeto foi reformulado, passando a ser: “Cogumelos comestíveis da Mata Atlântica: taxonomia integrativa, estudos etnomicológicos e viabilidade de cultivo”.

As áreas amostradas neste estudo correspondem a fragmentos de Mata Atlântica, com predominância nos estados das regiões Sul e Sudeste do Brasil. Complementarmente, foram incluídas coletas pontuais realizadas nas regiões Centro-Oeste e Nordeste, bem como no

Paraguai. O estudo também incorporou materiais provenientes de coleções depositadas nos seguintes herbários e fungários: FIFUNGI, FLOR, ICN, SP e UFRN - Fungos.

Os capítulos de resultados estão redigidos em língua inglesa, formatados conforme as normas dos periódicos aos quais serão submetidos[1], e organizados da seguinte maneira:

CAPÍTULO 1: intitulado "*Macrolepiota capelariae* (Agaricaceae, Basidiomycota): a new species from the Brazilian Atlantic Rainforest with extended records to Argentina and Mexico", descreve uma nova espécie de *Macrolepiota* seção *Macrolepiota* com base na morfologia e análise molecular da região ITS, consistindo de um artigo já publicado na revista *Phytotaxa* em 2022.

CAPÍTULO 2: intitulado "Documenting the diversity, edibility, and nutritional value of *Macrolepiota* mushrooms from Brazil with the proposal of three new edible species based on morphotaxonomy, multigene phylogenetic analyses and ethnomycological evidence", consiste em um artigo completo, preparado para submissão ao periódico *Studies in Mycology*. Neste trabalho, três espécies novas e duas variedades novas são propostas para o gênero *Macrolepiota* com base na morfologia e em dados moleculares multilocus (ITS, LSU, *rpb2* e *tef1-α*), além de fornecer dados adicionais sobre morfologia, filogenia e distribuição geográfica de espécies previamente conhecidas. Também, o manuscrito reúne dados etnomicológicos relacionados a cinco espécies e apresenta o perfil nutricional completo de três delas.

CAPÍTULO 3: intitulado "Unroughing the cat's tongue mushrooms: Four new species of *Pseudohydnum* from Brazil based on morphological and molecular phylogenetic evidence", descreve quatro espécies novas espécies de *Pseudohydnum* com base na morfologia e em dados moleculares multilocus (ITS, LSU e *rpb2*). Além disso, aborda o registro de comestibilidade de uma das espécies descritas, consistindo de um artigo já publicado na revista *Mycologia* em 2024.

CAPÍTULO 4: intitulado "A glimpse into the tropical underground: morphology and multigene phylogeny of true truffles (*Tuber*, Ascomycota) collections from Brazil reveal a new species and three new records", constitui um manuscrito elaborado segundo as normas do periódico *Mycologia*, ao qual será submetido para publicação. O trabalho aborda a descrição de uma nova espécie de trufa verdadeira (gênero *Tuber*) associada a árvores exóticas europeias e bem adaptada às condições climáticas do Sudeste brasileiro. Adicionalmente, três espécies do gênero *Tuber* são reportadas pela primeira vez para o território brasileiro, das quais duas constituem os primeiros registros documentados para o hemisfério sul. A investigação apoiou-

[1] Exceto os capítulos 1 e 3 que representam artigos completos já publicados.

se na caracterização morfológica aliada a inferências filogenéticas multilocus a partir dos seguintes marcadores moleculares: ITS, LSU, *rpb2* e *tef1-α.*

CAPÍTULO 5: intitulado “Taming the giant: first successful cultivation and nutritional composition of *Macrocybe titans* in Brazil”, consiste em um manuscrito elaborado com base nas diretrizes do periódico *IMA Fungus*, ao qual será submetido para publicação. Este trabalho representa o primeiro relato científico do cultivo bem-sucedido de uma linhagem silvestre de *Macrocybe titans* do Brasil, integrando também a análise de sua composição nutricional.

Com exceção dos Capítulos 1 e 3, os resultados apresentados nesta tese não têm como objetivo validar formalmente os nomes dos táxons propostos, mas apenas apresentá-los como parte dos achados obtidos, sem conferir-lhes, portanto, status nomenclatural neste momento. As espécies, variedades ou demais categorias taxonômicas sugeridas como novas não devem ser consideradas validamente publicadas, conforme estabelecido pelos Artigos 30.8 e 36.1 do *International Code of Nomenclature for algae, fungi, and plants* (Turland *et al*., 2018). Cabe destacar, ainda, que os autores das espécies mencionadas na introdução deste trabalho não serão indicados na primeira ocorrência dos nomes científicos. Essa convenção foi adotada exclusivamente nos artigos que compõem os capítulos.

As sequências geradas neste estudo, com exceção daquelas apresentadas nos Capítulos 1 e 3, ainda não foram submetidas ao GenBank para depósito, sendo apresentadas nos manuscritos ainda não publicados apenas pelo número do coletor e pelo nome atribuído ao táxon correspondente.

Por fim, vale ressaltar que todas as citações no corpo do texto desta tese estão destacadas em negrito. Embora essa prática não seja prevista pelas normas formais de formatação acadêmica (ex.: ABNT), o uso do negrito facilita a identificação rápida das referências durante a revisão, auxilia na conferência com a lista bibliográfica e contribui para uma leitura mais fluida em meio digital. Ciente de que esse recurso pode comprometer a formalidade do texto, seu uso está restrito à presente versão de trabalho.

Introdução geral

O reino Fungi representa um dos mais abundantes e diversos grupos de organismos que compõem a árvore da vida (Blackwell *et al*., 2012), ocorrendo em praticamente todos os ecossistemas terrestres como parceiros mutualistas, patógenos, parasitas ou sapróbios. Os fungos ocupam a segunda posição no ranking de diversidade biológica entre os eucariotos, perdendo apenas para os insetos (Niskanen *et al*., 2023). O número estimado de espécies fúngicas varia de 2,2 a 3,8 milhões, das quais apenas cerca de 155 mil espécies foram catalogadas cientificamente (Hawksworth & Lücking, 2017; Niskanen *et al*. 2018; Lucking *et al*. 2021). Além disso, os dados de *metabarcoding* indicam um número ainda mais expressivo de espécies, com estimativas que variam de 11,7 a 13,2 milhões (Wu *et al*., 2019). Nessa perspectiva, ao longo de mais de dois séculos de micologia, apenas uma pequena fração das espécies estimadas de fungos foram descritas, colocando esses organismos entre os menos conhecidos do planeta, juntamente com os insetos e bactérias (Wu *et al*., 2019).

A lacuna no conhecimento da diversidade de fungos se deve a uma combinação de fatores metodológicos, taxonômicos e estruturais. Um dos principais desafios é a dificuldade de detecção e cultivo de muitas espécies, especialmente os chamados *dark taxa*, que apresentam estruturas morfológicas de difícil isolamento e só podem ser identificados por sequenciamento ambiental (Nilsson *et al*., 2019; Ryberg & Nilsson, 2018). Além disso, a dependência de características morfológicas e de marcadores genéticos limitados, como a região ITS, dificulta a distinção de espécies crípticas (Bickford *et al*. 2007; Nilsson *et al*., 2019; Lücking *et al*., 2020). A escassez de especialistas em micologia e a distribuição desigual dos esforços de pesquisa, sobretudo em regiões tropicais ricas em biodiversidade, agravam essa situação (Blackwell, 2011).

As regiões tropicais e de clima quente parecem apresentar uma riqueza significativa em termos de diversidade fúngica ainda não explorada (Hyde *et al*., 2018; Menolli e Sanchez-Garcia, 2020). Entretanto, o aspecto mais crítico da estimativa da diversidade fúngica é a definição precisa de uma espécie (Chethana *et al*., 2021; Lücking *et al*., 2021). Portanto, a fim de determinar uma quantidade realista dos fungos existentes, faz-se necessário o aprofundamento das investigações acerca da biodiversidade global (Wu *et al.*, 2019). Nesse contexto, diversos métodos têm sido desenvolvidos e utilizados para identificar e descrever as espécies de fungos.

O reconhecimento e nomeação de espécies é um processo longo e aparentemente interminável, com revisões frequentes dos esquemas taxonômicos e de sistemática. Considerando a taxa média atual de aproximadamente 2.000 novas espécies descritas por ano,

seriam necessários pelo menos 2.100 anos para descrever a estimativa superior de 3,8 milhões de espécies de fungos proposta por Hawksworth e Lücking (2017) (Cornish, 2024). Nas últimas décadas, diferentes conceitos de espécies e métodos de delimitação têm sido amplamente utilizados para estudar as relações entre espécies intimamente relacionadas a fim de determinar os limites interespecíficos ou descrever organismos como novas espécies (Taylor *et al*. 2000; Lücking *et al*., 2020; Aime *et al*. 2021; Bhunjun et al. 2021; Crous *et al*. 2021). Nos últimos anos, tem se tornando cada vez mais evidente que a certificação da identificação dos fungos exige uma abordagem composta de várias etapas, a fim de gerar dados precisos e valiosos. Consequentemente, uma abordagem polifásica tem sido aplicada recentemente para identificar e caracterizar fungos de forma consistente. Essa abordagem, referida comumente como taxonomia integrativa, não é apenas uma simples combinação de métodos, mas uma síntese complexa de diferentes técnicas, cada uma contribuindo com uma perspectiva única (Dayrat 2005; Maharachchikumbura *et al*. 2021; Stengel *et al*. 2022). Essas técnicas incluem os estudos micro- e macromorfológicos, que se concentram nas características fenotípicas dos fungos; análises bioquímicas, que se aprofundam na composição química; e a biologia molecular, que examina o material genético e fornece dados para a inferência de filogenias (Lücking *et al*., 2020).

Em nível molecular, a região do Espaçador Interno Transcrito (ITS) continua sendo o *barcoding* universal para identificar as linhagens filogenéticas dos fungos, sendo usado, em muitos casos, como um primeiro diagnóstico para identificação (Nilsson *et al*., 2008; Schoch *et al*., 2012). Quando a região ITS não é suficiente para discriminar as espécies, é necessário empregar estratégias secundárias para atingir o nível desejado de precisão e exatidão (Lücking *et al*., 2020). Essa limitação é especialmente evidente na presença de complexos de espécies, conjuntos de táxons filogeneticamente próximos cuja delimitação permanece incerta e que frequentemente incluem espécies crípticas (Singh *et al*., 2015). Estas, por sua vez, correspondem a linhagens geneticamente distintas que compartilham morfologia praticamente idêntica, dificultando sua separação por métodos tradicionais baseados em caracteres morfológicos (Peintner *et al*., 2019). A baixa divergência na região ITS nesses grupos compromete a acurácia das delimitações taxonômicas e pode ocultar uma diversidade evolutivamente relevante. Essa situação tem sido amplamente documentada em diversos gêneros fúngicos, como *Fusarium*, *Trichoderma*, *Tuber*, *Armillaria* e *Penicillium* (Baturo-Cieśniewska *et al*., 2020; Leonardi *et al*., 2021). Nesses contextos, o uso de marcadores adicionais como rpb1, *rpb2*, *Tef1-α* e *β-tubulina*, aliado a métodos filogenéticos integrativos e estratégias baseadas em coalescência, tem se mostrado essencial para a correta identificação e reconhecimento de espécies (Singh *et al*., 2015; Lücking *et al*., 2020). O modo como os

marcadores individuais delimitam a circunscrição molecular das espécies é determinado pelo contexto, e a viabilidade de marcadores específicos não deve ser transferida sem críticas de um grupo taxonômico para outro, mas sim explorada empiricamente para cada táxon. Por fim, a filogenômica pode ser empregada para resolver complexos de espécies particularmente difíceis, mas essa abordagem exige grandes recursos computacionais e pessoais, e atualmente está limitada a estudos de casos exemplares (Lücking *et al*., 2020).

Macrofungos e Cogumelos Comestíveis

Os fungos que produzem cogumelos, amplamente conhecidos como macrofungos, pertencem majoritariamente ao filo Basidiomycota, embora também existam representantes em Ascomycota e, em menor proporção, entre os chamados fungos zigospóricos, sendo caracterizados pela produção de esporomas visíveis a olho nu e que crescem comumente acima do solo (epígeos) ou no subterrâneo (hipógeos) (Chang & Miles, 2004; Moore *et al*., 2011). Os cogumelos podem apresentar diversas formas, cores e tamanhos, variando desde a clássica configuração agaricoide com píleo, lamelas e estipe (ex.: *Amanita* spp. *Agaricus* spp.), até a formação de uma estrutura boletoide (ex.: *Suillus* spp., *Xerocomus* spp.), coraloide/clavarioide (ex.: *Clavulina* spp., *Ramaria* spp.), faloide (ex.: *Mutinus* spp., *Phallus* spp.), gelatinosa (ex.: *Auricularia* spp., *Tremella* spp.), gasteroide (ex.: *Amylascus* spp., *Tuber* spp.), hidnoide (ex.: *Hydnum* spp., *Pseudohydnum* spp.) ou poliporoide (ex.: *Ganoderma* spp., *Phellinus* spp.), dentre outras (Kirk *et al*., 2008; Nagy *et al*., 2017). Estima-se que cerca de 412–713 mil espécies de macrofungos ocorram globalmente, das quais apenas 29 mil (4–7%) têm sido cientificamente catalogadas (Hawksworth & Lücking, 2017). Dentre os Basidiomycota, a classe Agaricomycetes, com cerca de 21.000 espécies descritas, abriga o maior número de espécies formadoras de cogumelo, abrangendo diversos gêneros que figuram dentre os 100 mais citados com base em análises bibliométrica, tais como *Agaricus*, *Amanita*, *Ganoderma*, *Lactarius, Lentinula, Pleurotus* e *Russula* (He *et al*., 2019; Bhunjun *et al*., 2024). Enquanto em Ascomycota, os macrofungos estão distribuídos principalmente nas classes Leotiomycetes, Pezizomycetes e Sordariomycetes (Wijayawardene *et al*., 2017). Dentre os gêneros de macrofungos ascomicetos, *Tuber* (verdadeiras trufas) e *Morchella* (*morels* verdadeiros) são os de maior relevância alimentícia e comercial (Boa, 2004; Hyde *et al*., 2024).

Quanto ao uso e sua relação com os seres humanos, os cogumelos podem ser agrupados em quatro categorias: (1) cogumelos comestíveis (ex.: *Agaricus bisporus*, *Calocybe indica* e *Macrolepiota procera*); (2) cogumelos medicinais (ex.: *Ganoderma lucidum*, *Hericium erinaceus* e *Trametes versicolor*); (3) cogumelos tóxicos (ex.: *Amanita phalloides*, *Galerina*

marginata, *Cortinarius orellanus*); (4) cogumelos psicoativos (ex.: *Gymnopilus luteofolius*, *Panaeolus cyanescens*, *Psilocybe cubensis*), e (5) outros cogumelos, que incluem um grande número de espécies cujas propriedades ainda não estão bem definidas. Além disso, existem alguns cogumelos multifuncionais que possuem qualidades nutricionais, tônicas e medicinais (El Sheikha & Hu, 2018; Elkhateeb *et al*., 2022). A comestibilidade de uma espécie de cogumelo pode ser definida por parâmetros que incluem a ausência de efeitos tóxicos para os seres humanos e a presença de sabor e aroma agradáveis (Rubel & Arora, 2008). Contudo, é importante destacar que grande parte desse conhecimento não se origina exclusivamente da experimentação científica moderna, mas sim de práticas milenares fundamentadas na observação empírica e na transmissão oral entre diferentes povos e comunidades (Ruan-Soto & Ordaz-Velázquez, 2015; López-García *et al*., 2024). Assim, o saber etnomicológico, construído a partir do uso tradicional e do reconhecimento sensorial e simbólico dos cogumelos, constitui a base histórica daquilo que hoje entendemos como comestibilidade (Boa, 2004). Estudos recentes trazem o número de 2.189 espécies de cogumelos comestíveis (incluindo espécies também medicinais) em todo o mundo, das quais 2.006 podem ser consumidas com segurança, enquanto cerca de 183 requerem algum tipo de pré-tratamento ou são associadas a reações adversas em algumas pessoas (Li *et al*., 2021).

Em relação ao total de espécies de cogumelos comestíveis e ao número de registros de comestibilidade associados a cada país, observa-se uma variação expressiva, apresentando maior robustez em países com uma comunidade de pesquisa bem estabelecida, não apenas em micologia, mas também em silvicultura e áreas afins, onde os cogumelos desempenham um papel econômico relevante. Por exemplo, mais de 40% dados sobre comestibilidade foram obtidos de apenas três países: China (26,8%), Japão (7,4%) e México (6,7) (Li *et al*., 2021). Tal representação desproporcional não sugere necessariamente que esses países tenham uma grande diversidade de cogumelos comestíveis, mas principalmente que eles têm uma forte cultura em torno de seu uso, documentação e pesquisa. Dentre os gêneros que apresentam o maior número de espécies comestíveis, destacam-se, respectivamente, *Russula*, *Amanita* e *Lactarius* (Li *et al*., 2021). Contudo, ao se levar em conta a proporção entre o número de espécies comestíveis e o total de espécies descritas no gênero, os grupos mais representativos seriam para os gêneros *Auricularia*, *Retiboletus*, *Sparassis* e *Termitomyces*, nos quais as espécies comestíveis somam mais de 50% de todas as espécies conhecidas para o gênero (Li *et al*., 2021).

Do ponto de vista nutricional, os cogumelos comestíveis destacam-se pelo seu perfil rico e diversificado em macro e micronutrientes, sendo fontes significativas de proteínas de alto valor biológico, fibras alimentares, minerais essenciais (como Se, K, P e Cu) e compostos antioxidantes (Mattila *et al*., 2001; Valverde *et al*., 2015; Rathore *et al*., 2017). Sob o ponto de

vista nutracêutico, os cogumelos são valiosos por serem isentos de glúten e colesterol, além de conterem substâncias orgânicas, tais como tocoferóis e carotenoides, que contribuem para a proteção contra o desenvolvimento de lesões ateroscleróticas (Podkowa *et al*., 2021). De acordo com Samsudin & Abdullah (2019), os cogumelos fornecem tanto carboidratos digeríveis (ex.: trealose, manitol, glicogênio e glicose) quanto carboidratos não digeríveis (ex.: mananos, quitina e β-glucanos), sendo esses últimos responsáveis por conferir aos cogumelos propriedades funcionais adicionais, como modulação do sistema imunológico e efeitos antioxidantes (Łysakowska *et al*., 2023). Ademais, os cogumelos são considerados suplementos vitamínicos em potencial, pois são uma ótima fonte de vitaminas do complexo B (principalmente B_1, B_2, B_3, B_6 e B_9), além de serem a única fonte não animal de vitamina D (Heleno *et al*., 2010; Rathore *et al*., 2017; Cardwell *et al*., 2018). Estudos indicam que o consumo de 100 g de cogumelos pode fornecer entre 2% a 34% da ingestão diária recomendada de vitaminas do complexo B, variando conforme a espécie de cogumelo e o seu método de preparo (Podkowa *et al*., 2021).

Para além do valor nutricional, os cogumelos comestíveis são utilizados há muito tempo no tratamento de vários tipos de doenças. Muitas das espécies comumente consumidas possuem propriedades terapêuticas e são utilizadas para fins medicinais (Elkhateeb *et al*., 2022). Pelo menos 270 espécies de cogumelos comestíveis apresentam um potencial terapêutico que pode contribuir para o bem-estar humano (El Sheikha & Hu, 2018). Estima-se que existam mais de 100 propriedades medicinais atribuídas a esses organismos, e os principais usos medicinais incluem funções antioxidantes, anticancerígenas, antidiabéticas, antialérgicas, imunomoduladoras, protetoras cardiovasculares, anticolesterolêmicas, antivirais, antibacterianas, antiparasitárias, antifúngicas, de desintoxicação e hepatoprotetoras (Persson, 2016; Rathore *et al*., 2017; Yahaya *et al*., 2017; Song *et al*., 2020; Elkhateeb *et al*., 2022). Além disso, foi demonstrada ação de cogumelos na proteção contra o desenvolvimento de tumores e processos inflamatórios (Hetland *et al*., 2020; Górska-Jakubowska *et al*., 2025). O entendimento para esse amplo espectro de propriedades medicinais dos cogumelos está no vasto arsenal de composto bioativos encontrados no esporoma e no micélio cultivado, tais como polissacarídeos, proteínas, gorduras, minerais, glicosídeos, alcaloides, óleos voláteis, terpenoides, tocoferóis, compostos fenólicos, flavonoides, carotenoides, folatos, lectinas, enzimas, ácidos ascórbicos e compostos orgânicos em geral (Patel & Goyal, 2012; Shang *et al*., 2015; Valverde *et al*., 2015; Rai *et al*., 2021).

Ambientalmente, os cogumelos desempenham funções ecológicas vitais, atuando como agentes decompositores, simbiontes micorrízicos, parasitas e mantenedores da biodiversidade (Bahram & Netherway *et al*., 2022). Espécies sapróbias promovem a decomposição de resíduos

lignocelulósicos, contribuindo significativamente para a ciclagem de nutrientes e o enriquecimento da matéria orgânica no solo (Orwin *et al*. 2011; Crowther *et al*. 2012; Nigo *et al*. 2023). Em paralelo, muitas espécies estabelecem associações simbióticas com raízes de plantas, formando ectomicorrizas. Nessas relações, em troca dos carboidratos produzidos pela planta por meio da fotossíntese, as espécies de cogumelo aumentam a capacidade da planta de absorver água e nutrientes do solo (Ogwu *et al*., 2025). Essa relação simbiótica é considerada crucial para uma vasta gama de espécies vegetais, sendo fundamental para sua sobrevivência, crescimento e resiliência em diversos ecossistemas ao redor do mundo (Ogwu *et al*., 2025). De fato, essas redes fúngicas podem conectar as raízes de diferentes espécies de plantas, promovendo uma interdependência natural e aumentando significativamente a diversidade do microbioma do solo (Rillig *et al*., 2024). Quando conectadas dessa forma, essas redes são conhecidas como redes micorrízicas comuns (do inglês, *Common Mycorrhizal Networks* – CMNs), e são essenciais para a resiliência dos sistemas botânicos, pois facilitam o compartilhamento e a redistribuição de água e nutrientes por toda uma comunidade de espécies vegetais (Horlick, 2023; Rillig *et al*., 2024). Os cogumelos também fornecem micro-*habitats* para uma ampla variedade de organismos, desempenhando papel estruturante nas cadeias alimentares e ampliando a complexidade ecológica dos *habitats* onde ocorrem (Bahram & Netherway *et al*. 2022; Kolényová *et al*. 2024).

Além de seu papel tradicional como fonte alimentar e medicinal, os cogumelos possuem grande potencial na biorremediação e no enfrentamento das mudanças climáticas. Diversas espécies de fungos, especialmente os basidiomicetos ligninolíticos, têm demonstrado capacidade de degradar compostos tóxicos e poluentes orgânicos persistentes, como hidrocarbonetos aromáticos policíclicos (HAPs), pesticidas e até metais pesados, por meio de processos enzimáticos oxidativos (Harms *et al*., 2011; Pointing, 2001). Essa estratégia, conhecida como micorremediação, vem sendo explorada como uma alternativa ecológica para a descontaminação de solos e corpos d'água (Harms *et al*., 2011; Pointing, 2001). No contexto das mudanças climáticas, os fungos micorrízicos desempenham papel crucial no sequestro de carbono no solo (Clemmensen *et al*., 2013). Estima-se que redes micorrízicas associadas a raízes vegetais possam contribuir com até 70% da biomassa subterrânea em ecossistemas como florestas boreais, promovendo o acúmulo de carbono estável no solo e auxiliando na regulação do ciclo global do carbono (Clemmensen *et al*., 2013).

Cogumelos Comestíveis Silvestres no Brasil

No Brasil, por muito tempo, o conhecimento acerca da diversidade e uso dos cogumelos comestíveis silvestres permaneceu negligenciado e bastante fragmentado na literatura científica (Góes-Neto & Bandeira, 2003; Cardoso *et al*., 2010; Vargas-Isla *et al*., 2013; Drewinski *et al.*, 2024a). O número e a confiabilidade de diversos registros de comestibilidade são limitados principalmente por problemas que envolvem a correta identificação das espécies. Tais problemas são evidenciados, por exemplo, pela aplicação generalizada de epítetos de espécies nativas da América do Norte e Eurásia, dentre outras regiões, a espécies da região neotropical, o que por muito tempo mascarou o conhecimento acerca das espécies comestíveis nativas do Brasil e de outras partes da América do Sul e Central. Graças ao uso das ferramentas moleculares, estamos testemunhando, desde o início da última década, um rápido aumento no número de cogumelos comestíveis sendo identificados por meio de uma abordagem taxonômica integrativa; no entanto, ainda poucas investigações têm acoplado esse enfoque polifásico aos estudos etnomicológicos (Sanuma *et al*., 2016; Drewinski *et al.*, 2024a).

Recentemente, com base em novas amostragens, revisão de registros bibliográficos e buscas no repositório GenBank (https://www.ncbi.nlm.nih.gov/genbank/), Drewinski *et al.* (2024a) compilaram uma lista de 409 espécies de cogumelos comestíveis silvestres para o Brasil, das quais 350 podem ser consumidas com segurança e 59 têm alguma restrição no seu uso como alimento. O estudo em apreço indica, ainda, que outras 150 espécies representam táxons cujas evidências de consumo ou *status* de comestibilidade se encontram pouco claras ou não foram confirmadas. Por fim, os autores chegaram a confirmar a identidade e a ocorrência no país de 86 espécies comestíveis da lista inicial, com base em dados moleculares e/ou tipos nomenclaturais brasileiros. Para as outras 323 espécies restantes, o estudo endereça a necessidade de investigações taxonômicas mais detalhadas para confirmar sua identidade e ocorrência no país, como é o caso, por exemplo, das espécies dos gêneros *Macrolepiota*, *Pseudohydnum* e *Tuber* citada para o país.

Etnomicologia e o Consumo de Cogumelos Comestíveis

O uso de cogumelos na alimentação humana remonta à Pré-História. Evidências arqueológicas encontradas na caverna de El Mirón, na Espanha, indicam que caçadores-coletores do Paleolítico Superior (18.000–12.000 anos atrás) consumiam fungos, conforme análise de resíduos em cálculo dentário (Power *et al*., 2015). Outro exemplo marcante é o "Homem de Ötzi", datado de cerca de 5.300 anos atrás, cuja população se utilizava de cogumelos, como *Fomes fomentarius* e *Fomitopsis betulina* (anteriormente conhecida

como *Piptoporus betulinus*), para usos medicinais ou utilitários, como iscas para acender fogo (Peintner *et al*., 1998; Stamets & Zwickey, 2014).

Na Antiguidade, os cogumelos ganharam importância tanto na alimentação quanto na medicina. Na China, o *Ganoderma lucidum*, conhecido como "Lingzhi" ou "Reishi", já era descrito no clássico da medicina herbal "Shennong Bencao Jing", por volta do século I d.C., como um remédio para promover longevidade e vitalidade (Valverde *et al*., 2015). No Antigo Egito, há registros hieroglíficos datados de cerca de 4.600 anos atrás que descrevem os cogumelos como alimentos sagrados, reservados exclusivamente aos faraós, e considerados "plantas da imortalidade", relacionados ao mundo espiritual (Chang *et al*., 2006)

Na Grécia clássica, Hipócrates (460–370 a.C.), considerado o pai da medicina ocidental, reconhecia os cogumelos como substâncias com propriedades anti-inflamatórias (Stamets & Zwickey, 2014). Já em Roma, Plínio, o Velho, escreveu sobre a dualidade dos cogumelos, ora fonte de prazer gastronômico, ora perigosos por seu potencial tóxico (Stamets & Zwickey, 2014). O tratado culinário "De Re Coquinaria", atribuído a Apício, também incluía receitas com cogumelos comestíveis (Stamets & Zwickey, 2014). Paralelamente, na América pré-colombiana, há forte evidência do uso ritualístico de cogumelos, onde povos mesoamericanos, como os maias e astecas, utilizavam espécies do gênero *Psilocybe* em cerimônias religiosas (Guzmán, 2008). Os astecas chamavam esses cogumelos de *teonanácatl*, "carne dos deuses", e acreditavam que eles permitiam a comunicação com divindades (Hofmann 1971; Carod-Artal, 2015). As práticas envolvendo o *teonanácatl* estavam profundamente inseridas na cosmologia e nos rituais religiosos dessas civilizações, sendo conduzidas por sacerdotes e xamãs que orientavam as experiências visionárias para fins de cura, adivinhação ou conexão espiritual (Hofmann 1971; Carod-Artal, 2015). Artefatos como as "pedras de cogumelo", datadas de aproximadamente 1000 a.C., corroboram esse uso ritualístico (Schultes *et al*. 2001; Guzmán, 2008; Carod-Artal, 2015).

A identificação de cogumelos comestíveis por humanos primitivos foi provavelmente um processo empírico e cumulativo, baseado em observação, tentativa e erro, e transmissão cultural. A observação de animais consumindo certos fungos sem efeitos adversos pode ter fornecido pistas iniciais sobre sua comestibilidade (Guzmán, 2008; Carod-Artal, 2015). Além disso, práticas como aplicar pequenas quantidades na pele ou consumir porções mínimas permitiam testar a toxicidade de forma relativamente segura (Guzmán, 2008; Carod-Artal, 2015). Ao longo do tempo, o conhecimento adquirido foi sendo transmitido oralmente entre gerações, consolidando uma sabedoria coletiva essencial à sobrevivência (Guzmán, 2008; Carod-Artal, 2015). A introdução do fogo e o desenvolvimento da culinária desempenharam um papel decisivo na ampliação da dieta humana. O cozimento de alimentos não apenas

melhorava o sabor e a digestibilidade, como também neutralizava compostos tóxicos presentes em várias espécies vegetais e fúngicas (Carmody & Wrangham, 2009).

No contexto atual, o reconhecimento da comestibilidade de cogumelos silvestres é resultado da intersecção entre o conhecimento científico e o saber tradicional, sendo este último uma fonte histórica e essencial na identificação de espécies seguras para o consumo humano (Boa, 2004). Comunidades rurais e populações tradicionais acumulam, ao longo de gerações, informações empíricas significativas acerca de espécies locais comestíveis, modos de preparo e potenciais efeitos adversos, formando uma base etnomicológica indispensável (Dar *et al*., 2023; Ríos-García *et al*., 2023). Em paralelo, esses saberes têm sido progressivamente integrados e validados por meio de metodologias taxonômicas contemporâneas, análises morfológicas e, mais recentemente, técnicas moleculares e estudos micoquímicos, que confirmam ou revisam a segurança alimentar das espécies envolvidas (Adeniyi *et al*., 2018; Hussain *et al*., 2023). A literatura científica, os bancos de dados micológicos e as pesquisas toxicológicas complementam esse processo, fornecendo critérios técnicos para a diferenciação entre espécies comestíveis, tóxicas e potencialmente letais (De Roman *et al*., 2006). Apesar disso, o conhecimento tradicional continua a ser um ponto de partida confiável e culturalmente enraizado para a seleção de cogumelos comestíveis, especialmente em contextos de elevada diversidade micológica, como observado em várias regiões tropicais e subtropicais (Boa, 2004; De Roman *et al*., 2006).

A estudos etnomicológicos no Brasil tiveram seu marco inicial com os trabalhos pioneiros de Oswaldo Fidalgo, que foi o primeiro a propor uma abordagem sistemática sobre os conhecimentos micológicos entre povos indígenas. Fidalgo (1965) reuniu registros etnográficos sobre o uso alimentar e o conhecimento empírico de cogumelos entre povos indígenas como os Tupinambá, Carajá, Bororo e Kaingang, contestando a ideia predominante da micofobia indígena. Posteriormente, em 1979, as investigações etnomicológicas foram ampliadas para outras etnias da região do Xingu (Mato Grosso), como os Caiabi, Txicão e Txucarramãe, documentando em detalhe os sistemas vernaculares de classificação, os critérios de comestibilidade e as práticas culinárias associadas aos macrofungos (Fidalgo & Hirata, 1979). Nesse estudo, foram mencionados e analisados 26 termos micológicos utilizados por tais etnias. Ademais, o consumo de pelo menos duas espécies de cogumelo, *Lentinus crinitus* e *Auricularia fuscosuccinea*, foi confirmado na etnia Txicão, que os preparavam de maneira simples, sendo envoltos em folhas verdes e assados sobre cinzas ou brasas, refletindo práticas culinárias tradicionais (Fidalgo & Hirata, 1979).

Paralelamente, Sir Ghillean T. Prance, etnobotânico britânico com ampla atuação na Amazônia, também desempenhou um papel fundamental na consolidação da etnomicologia

como campo de estudo no Brasil (Prance, 1972, 1973, 1984, 1986; Fidalgo & Prance, 1976). Sua primeira contribuição direta ocorreu em 1971, durante uma expedição ao noroeste do estado de Roraima, onde conduziu levantamentos etnobotânicos entre diferentes comunidades indígenas (Prance, 1972). Nesse estudo pioneiro, Prance observou que, entre os grupos visitados, apenas os Waiká (subgrupo Yanomami) faziam uso regular de cogumelos na alimentação, e foram registradas quatro espécies fúngicas utilizadas como alimento: *Favolus brasiliensis*, *F. tessellatus*, *Polyporus stipitarius* e *Neoclitocybe byssiseda* (Prance, 1972). Posteriormente, Fidalgo & Prance (1976) descreveram a complexidade do saber micológico Sanöma (também Yanomami), com destaque para os sistemas de nomenclatura, distinções entre espécies comestíveis e tóxicas, bem como o profundo conhecimento ecológico local sobre sazonalidade e habitat dos fungos. Nesse estudo, foram registradas 21 espécies de cogumelos comestíveis silvestres consumidas por este grupo do povo Yanomami.

A partir do início da década de 2010, apenas três estudos etnomicológicos foram realizados junto a comunidades indígenas da Amazônia: (1) Vargas-Isla *et al*. (2013), (2) Sanuma *et al*. (2016) e (3) Yanomami *et al*. (2019). Esses trabalhos consolidaram uma fase da etnomicologia brasileira marcada por um viés mais cognitivo, cuja origem é atribuída aos estudos de Fidalgo, conforme argumentado por Góes-Neto & Bandeira (2003). Os estudos com viés cognitivista buscaram registrar conhecimentos além dos usos, como nomes, taxonomia popular, percepções ecológicas e a cosmovisão associada aos fungos (Trierveiler-Pereira *et al*., 2022). Em paralelo, observa-se o florescimento de um novo período dos estudos etnomicológicos brasileiros, em que os trabalhos passaram por um processo de diversificação, tanto em termos de abordagem como na ampliação dos grupos sociais estudados. Tradicionalmente concentrada em populações indígenas, cuja cosmovisão e práticas de uso de recursos naturais sempre foram valorizadas, a etnomicologia brasileira passou a investigar também os conhecimentos de comunidades não indígenas, como populações rurais, quilombolas, caiçaras e agricultores familiares (Trierveiler-Pereira *et al*., 2022). Essa nova fase tem sido referida como: Período da Diversidade, e tem como principal marco os trabalhos de Sousa *et al*. (2015, 2017a, b). Esses trabalhos se diferenciam não apenas pela ênfase regional no Nordeste brasileiro, mas, sobretudo, pela escolha inédita da comunidade investigada. Tratam-se dos primeiros estudos etnomicológicos no Brasil voltados a comunidades rurais, inaugurando uma nova vertente de pesquisa e ampliando as fronteiras do conhecimento sobre as relações entre os saberes tradicionais e os macrofungos. Posteriormente, outros estudos dedicaram-se igualmente a investigar o uso alimentar ou medicinal dos macrofungos em comunidades rurais e urbanas em diferentes regiões do Brasil: Santos *et al*. (2020), Santana *et al*. (2020, 2022), Andrade *et al*. (2021), Calaça *et al*. (2021), Silva *et al*. (2022), Prado-Elias *et*

al. (2022, 2024), Sousa *et al*. (2024). Nesse contexto, ainda pouco estudos têm trazido informações sobre o uso de cogumelos comestíveis em comunidades rurais e urbanas nas regiões Sul e Sudeste.

O consumo de cogumelos comestíveis no Sudeste do Brasil está fortemente associado às tradições culturais de comunidades de imigrantes e populações locais, que identificaram o potencial alimentício e terapêutico de espécies fúngicas nativas e exóticas (Prado-Elias *et al*. 2022). Historicamente, destaca-se o papel central dos imigrantes orientais, em especial os japoneses e chineses, na introdução e desenvolvimento da micocultura na região (Bett & Perondi, 2011). No estado de São Paulo, registros indicam a comercialização e o consumo de espécies silvestres, como *Ramaria toxica* (citada como *R. flavobrunnescens*), em comunidades japonesas da região metropolitana da cidade de São Paulo (Fidalgo & Fidalgo, 1970), bem como o início do consumo e domesticação de *Agaricus subrufescens* (Cogumelo-do-sol ou "Cogumelo medicinal") por um imigrante japonês na cidade de Piedade (Dias *et al*., 2004). Ainda no contexto paulista, os imigrantes alemães também trouxeram consigo o hábito de consumir cogumelos. A imigração alemã no distrito de Parelheiros, localizado na zona sul da cidade de São Paulo, teve início no final do século XIX e início do século XX, quando diversas famílias se estabeleceram na região em busca de melhores condições de vida e oportunidades agrícolas (Prefeitura Municipal de São Paulo, 2019). Em meio à Mata Atlântica, os imigrantes germânicos passaram a cultivar hortaliças, criar animais e aproveitar os recursos naturais, dentre eles os cogumelos silvestres que se assemelhavam com espécies comestíveis típicas do continente europeu (Coelho-Nascimento & Silva, "com. pess.", 2025). Estudos etnomicológicos mais recentes identificaram o uso alimentar de espécies silvestres comestíveis, como *Neofavolus subpurpurascens* e *Phlebopus beniensis*, por comunidades rurais caboclas do estado de São Paulo (Trierveiler-Pereira, 2019; Prado-Elias *et al*., 2022).

No Sul do país, o consumo de cogumelos por comunidades descendentes de imigrantes europeus, como italianos, poloneses, russos e ucranianos, também foi registrado, embora ainda pouco explorado academicamente (Menolli *et al*., 2025). Há relatos de uso culinário de espécies como *Agaricus* cf. *arvensis* e *Auricularia fuscosuccinea* no Paraná (Meijer, 2001), além do consumo de *Macrolepiota bonaerensis*, por italianos na região de Curitiba (Meijer *et al*., 2007), e de espécies do gênero *Armillaria*, conhecidos localmente como "Pepenke-trigo" e "Pepenke-centeio", por ucranianos na região de Prudentópolis(Menolli *et al*., 2025). Ademais, em São Bento do Sul, no norte de Santa Catarina, região marcada pela colonização polonesa, Ludwinsky (2021) observou que descendentes desses imigrantes ainda mantêm a prática tradicional de coletar e consumir cogumelos silvestres, embora as espécies utilizadas não tenham sido identificadas.

Recentemente, Prado-Elias *et al*. (2025) documentaram o uso tradicional de cogumelos por comunidades descendentes de imigrantes eslavos, poloneses e ucranianos, na região do Planalto Norte de Santa Catarina, nos municípios de Itaiópolis e Papanduva. O estudo identificou 12 espécies silvestres utilizadas como alimento, trazendo informações sobre nomes vernaculares, formas de preparo, usos medicinais e práticas culturais associadas. Os resultados desses trabalhos destacam a riqueza do patrimônio etnomicológico regional e reforçam a importância dos estudos etnomicológicos como ferramentas essenciais para documentar práticas culturais em risco de desaparecimento, promover a valorização do conhecimento tradicional e fortalecer estratégias de conservação biocultural.

Cultivo de Cogumelos Comestíveis

Inicialmente os cogumelos eram colhidos apenas nas florestas, depois passaram a ser cultivados pelo homem (Aaronson, 2000). Registros apontam a colheita e o consumo de cogumelos silvestres nas civilizações grega e romana, especialmente pela população mais nobre (Buller, 1914).

Embora a colheita comercial de cogumelos silvestre continue hoje, a maior parte do suprimento mundial vem de produtores comerciais de cogumelos (Boa, 2004). Os chineses e japoneses têm sido elencados como pioneiros no cultivo profissional de cogumelos, sendo *Auricularia auricula-judae* (= *Auricularia auricula*), *Flamulina velutipes* e *Lentinula edodes* (Shiitake) as primeiras espécies a serem produzidas, respectivamente, por volta de 600, 800 e 1100 d.C., sempre após sucessivas décadas de esforços de domesticação (Ainsworth, 1976; Chang & Miles, 1987; Wu *et al*., 2014). Vale ressaltar que estudos recentes demonstraram que os cultivos asiáticos atribuídos à *A. auricula-judae*, originalmente descrita para a Europa, correspondem, na verdade, a uma nova espécie, *A. heimuer*, distinta e endêmica do Leste Asiático (Wu *et al*., 2014; Wu & Dai, 2015; Wu *et al*., 2021). Já na Europa, o cultivo do *Agaricus bisporus*, conhecido como *champignon*, teve início na França, por volta do século XVII, quando agricultores começaram a reproduzir as condições ideais de crescimento em ambientes subterrâneos, como adegas e cavernas (Boa, 2004). Esse avanço técnico marcou uma mudança significativa na relação humana com os fungos, consolidando uma prática que se expandiu globalmente com o tempo, não apenas com fins alimentares, mas também medicinais e biotecnológicos.

Embora cerca de 10% da diversidade atual de cogumelos seja comestível, apenas cerca de 25 espécies são cultivadas comercialmente e aproximadamente oito são mais frequentemente produzidas (Chang & Miles 2004; Royse *et al*., 2017) Os gêneros de cogumelos comestíveis

que mais se destacam no cenário do cultivo mundial são *Lentinula* (22%), *Pleurotus* (19%), *Auricularia* (18%), *Agaricus* (15%), *Flammulina* (11%), *Volvariella* (5%) e outros (10%), de acordo com os dados de Royse *et al*. (2017). As espécies desses gêneros são cultivadas em diferentes regiões do mundo, com destaque para a região Ásia-Pacífico, que liderou o mercado de cogumelos em 2023, com uma participação de 79,83% da produção global. (FBI, 2025). No mesmo ano, a China liderou a produção mundial de cogumelos, com um total de 47.14 Mt produzidas, seguida pelo Japão (462 t), pela Índia (315 t), pelos Estados Unidos (302 t) e pela Polônia (240 t) (FAO, 2025).

A produção global de cogumelos experimentou um crescimento exponencial desde 1961, quando era de 0,5 Mt, até atingir 34,3 Mt em 2013 (FAO, 2025). Atualmente, estima-se que a produção tenha superado 50 Mt (FAO, 2025), indicando um aumento acentuado no consumo *per capita* de cogumelos (Royse *et al*., 2017). Em 2022, o mercado global de cogumelos foi avaliado em US$ 56 bilhões, tendo aumentado para US$ 62 bilhões em 2023 (Deb, 2024). A projeção é de que o crescimento se mantenha, e o mercado seja definido para atingir o valor impressionante de US$ 136 bilhões até 2032 (Deb, 2024). Esse proeminente avanço tem gerado emprego e oportunidades empresariais, além de fornecer alimentos nutritivos e funcionais (Kapahi, 2018). Ademais, o cultivo de cogumelos tem sido visto como uma alternativa econômica e eficiente na biotransformação de resíduos florestais e agroindustriais em alimentos com alto teor de proteínas, reduzindo a poluição e promovendo a sustentabilidade (Pandey *et al*., 2000; Chiu & Moore, 2001; Dias *et al*., 2003; Bonatti *et al*., 2003, 2004; Singh, 2011).

A fungicultura no Brasil tem experimentado um crescimento significativo, acompanhando a tendência global de valorização dos cogumelos comestíveis como alimentos funcionais e sustentáveis (Gomes *et al.* 2016). Historicamente concentrada no estado de São Paulo, especialmente na região do Alto Tietê, a atividade consolidou-se como referência nacional, com destaque para municípios como Mogi das Cruzes, Ibiúna, Sorocaba, Valinhos, Juquitiba, Salto, Pinhalzinho e Cabreúva (Cabrera *et al*., 2020, ANPC, 2025). Além de São Paulo, o estado do Paraná vem se destacando na produção de cogumelos, especialmente nas cidades de Castro, Tijucas do Sul e Curitiba, onde o clima favorável e o incentivo à agricultura familiar favorecem o cultivo (ANPC, 2025). Outros estados também vêm incorporando essa atividade em suas cadeias agroindustriais, tais como Minas Gerais, Rio de Janeiro, Rio Grande do Sul, Bahia e Pernambuco, além do Distrito Federal (ANPC, 2025). Porém, a fungicultura brasileira ainda carece de estatísticas precisas sobre a produção em escala nacional, dificultando diagnósticos mais abrangentes. Mesmo assim, o cenário aponta para um aumento progressivo

da diversificação regional, com potencial de geração de renda, inclusão produtiva e fortalecimento de cadeias agroecológicas locais (ANPC, 2025).

O cultivo de cogumelos comestíveis no Brasil teve início na década de 1950, impulsionado principalmente por imigrantes japoneses que se estabeleceram na região do Alto Tietê, no estado de São Paulo, especialmente no município de Mogi das Cruzes (Abe, 1980). Esses imigrantes trouxeram consigo técnicas tradicionais de fungicultura, adaptando-as às condições locais e utilizando substratos disponíveis, como toras de madeira e resíduos agrícolas (Dias 2010; Abe, 1980). A primeira espécie de cogumelo cultivada comercialmente foi o *Agaricus bisporus* [como *A. campestris* ou *Psalliota campestris*], conhecido como *champignon* ou cogumelo-Paris) e cuja produção foi introduzida por imigrantes japoneses e italianos em Mogi das Cruzes e Atibaia, em 1953 (Bononi *et al*., 1999, Dias 2010), quase 100 anos após seu cultivo ser introduzido no continente Americano (Vedder, 1996). Posteriormente, outras espécies como o *Lentinula edodes* (*shiitake*) e o *Pleurotus ostreatus* (*shimeji*) foram incorporadas à produção local, aproveitando o conhecimento dos imigrantes asiáticos (Abe, 1980).

Atualmente, os estados de São Paulo e Paraná lideram a produção e o consumo de cogumelos cultivados no Brasil, com São Paulo sendo responsável por aproximadamente 80% da produção nacional (Minervino, 2024; ANPC, 2025). A produção anual de cogumelos no Brasil foi estimada em 12.730 t no Censo Agropecuário 2017 (IBGE, 2017), enquanto Sánchez *et al*. (2018) indicaram um volume superior, de 15.696 t, sugerindo a expansão do setor. As principais espécies cultivadas incluem *Agaricus bisporus*, *Lentinula edodes* e espécies do gênero *Pleurotus* (Gomes, 2013; ANPC, 2025). Dentre elas, '*Pleurotus ostreatus* var. *florida*' é a mais produzida, com cerca de 8.000 t anuais de cogumelos frescos; embora a espécie *Agaricus bisporus* seja a mais consumida no país, chegando a cerca de 15 mil toneladas ao ano (Menolli *et al*., 2025). Além disso, espécies medicinais como *Ganoderma lingzhi* (por vezes citada como *G. lucidum* e conhecida como Reishi, "cogumelo da imortalidade" ou Cogumelo-rei), *Hericium erinaceus* (Juba-de-leão ou cogumelo-pom-pom) e *Agaricus subrufescens* têm ganhado espaço em nichos especializados (Menolli *et al*., 2025). Atualmente, *Agaricus subrufescens* representa a única espécie cultivada em escala comercial no Brasil com produção voltada à exportação, totalizando cerca de 15 t de cogumelo desidratado por ano (Menolli *et al*., 2025). Já o cultivo de *G. lucidum* e *H. erinaceus* ainda é incipiente, porém promissor, com iniciativas voltadas à diversificação e agregação de valor no mercado de cogumelos especiais (Urben *et al*. 2017; Urben & Uriartt, 2017).

Dados do primeiro censo paulista de produção de cogumelos comestíveis e medicinais, realizado em 2016, revelaram a existência de 505 produtores distribuídos em 93 municípios do

estado de São Paulo, com uma produção mensal de cerca de 1.062 toneladas, gerando uma receita na ordem de cerca de R$ 21 milhões e aproximadamente 5.000 empregos diretos (Gomes *et al.*, 2016). Esses números revelam uma expansão significativa em relação ao levantamento de 2007–2008 pela LUPA/CATI/SP, que registrou 265 produtores em 44 municípios (Gomes *et al.*, 2016). Em âmbito nacional, o cultivo de cogumelos vem acompanhando essa expansão (Dias, 2010), cuja estimativa é de que 2.000 produtores rurais se dedicam à atividade no Brasil (Minervino, 2024). Nesse contexto, o cultivo de espécies exóticas desponta como um segmento promissor no país, impulsionado pelas mudanças no perfil dos consumidores, que acompanham a tendência global de valorização de alimentos com propriedades funcionais, produzidos de forma sustentável e com reduzido impacto ambiental (Hsu *et al*., 2020; Van Bussel *et al*., 2022; Nichifor *et al*., 2025). No entanto, diversos entraves comprometem a consolidação e a expansão do setor, mesmo diante do aumento da demanda (Dias, 2010).

No Brasil, o cultivo de cogumelos comestíveis ainda depende majoritariamente da adaptação de tecnologias originalmente desenvolvidas para espécies de clima temperado, p. ex. *A. bisporus*, *L. edodes*, e *Pleurotus* spp. A transposição desses sistemas produtivos para ambientes tropicais impõe desafios técnicos, como a necessidade de ambientes climatizados, maior controle fitossanitário e adequação de substratos, o que eleva os custos operacionais e dificulta a prática por pequenos produtores (Dias, 2010; Cabrera *et al*., 2020). Nesse cenário, a domesticação de espécies silvestres nativas surge como uma alternativa estratégica para a diversificação da fungicultura nacional. Essa abordagem pode favorecer a criação de sistemas de produção mais resilientes e adaptados às condições edafoclimáticas locais, além de fomentar a bioeconomia regional e estimular a conservação da diversidade fúngica ameaçada pela degradação ambiental (Albertó, 2017; Menolli *et al*., 2025). Ao integrar espécies nativas ao cultivo comercial, promove-se não apenas a inovação na cadeia produtiva de cogumelos, mas também a valorização do conhecimento tradicional associado ao uso desses recursos por comunidades locais.

A atual produção de cogumelos é baseada no processo de domesticação de isolados coletados na natureza nos últimos 50 anos e que foram selecionados por meio de estudos de avaliação da qualidade e produtividade (Albertó, 2017). Tais estudos têm contemplado predominantemente espécies que ocorrem em regiões temperadas, ao passo que existe pouca informação sobre cogumelos silvestres e sua viabilidade de cultivo em regiões tropicais (Klomklung *et al*., 2012; Thongklang *et al*., 2014). Dentro da vasta diversidade de macrofungos presentes nos ecossistemas naturais, apenas uma fração foi domesticada para fins alimentícios. Estima-se que cerca de 130 espécies de cogumelos já tenham sido domesticadas globalmente (Thongklang *et al*., 2014).O processo de domesticação de cogumelos silvestres envolve

múltiplas etapas, que vão desde o isolamento do micélio até o desenvolvimento de protocolos de cultivo e avaliação nutricional. Albertó (2017) descreveu esse processo em 14 etapas, incluindo a seleção de substratos e suplementações, condições de frutificação, avaliação de características morfológicas e testes pós-colheita. Apesar do potencial, os estudos brasileiros sobre a domesticação de cogumelos silvestres ainda são incipientes, mas alguns estudos têm contribuído significativamente para a consolidação de espécies nativas como candidatas à fungicultura comercial. Um exemplo pioneiro é o trabalho de Ruegger *et al*. (2001), que avaliou o cultivo de *Oudemansiella* sp. em substratos compostos por serragem de eucalipto e bagaço de cana-de-açúcar, com suplementação de farelo de trigo.

Desde então, diversas iniciativas de pesquisa têm se concentrado na identificação, isolamento e caracterização de cogumelos silvestres comestíveis nativos do Brasil, com o objetivo de avaliar seu potencial para o cultivo em condições controladas. Nesse contexto, espécies como *Auricularia cornea*, *Auricularia fuscossuccinea*, *Favolus tessellatus* (como *Polyporus tenuiculus*), *Irpex rosettiformis*, *Lentinus crinitus*, *Panus neostrigosus* (como *Lentinus strigosus* ou *Panus lecomtei*), *Panus strigellus*, *Pleurotus albidus*, *Pleurotus pulmonarius* e *Trametes sanguinea* (como *Pycnoporus sanguineus*) têm sido testadas em estudos de domesticação (Omarini *et al*., 2009; Sales-Campos & Andrade, 2011; Albertó & Omarini (2012); Vargas-Isla *et al*., 2015; Gambato *et al*., 2016; Castro-Alves *et al*., 2017; Bertéli *et al*., 2021; Chilanti *et al*., 2022; Colla *et al*., 2023; Drewinski *et al*., 2024b; Drewinski *et al*., 2024c; Drewinski *et al*., 2025).

Contudo, limitações ainda são observadas quanto à correta identificação taxonômica dos isolados silvestres utilizados em alguns estudos de cultivo. Como, por exemplo, a aplicação inconsistente do nome *Ganoderma lucidum* para cepas coletadas no Sul do Brasil (Rolim *et al*., 2014) ou de *Pleurotus ostreatus* para linhagens amazônicas (Aguiar *et al*., 2021; Sales-Campos *et al*., 2021; Aguiar *et al*., 2022), uma vez que essas espécies têm distribuição putativamente restrita ao Hemisfério Norte (Fryssouli *et al.*, 2020; Li *et al.*, 2020). Tais incongruências evidenciam a necessidade de análises filogenéticas rigorosas e confirmatórias, uma vez que a adoção da nomenclatura de espécies exóticas sem validação filogenética pode ocultar a diversidade genética real das espécies nativas, comprometendo tanto a conservação quanto o aproveitamento biotecnológico desses recursos. Outra limitação recorrente nos estudos brasileiros sobre domesticação de cogumelos silvestres é a baixa representatividade de espécies terrícolas ou micorrízicas. Até o momento, os únicos casos formalmente documentados referem-se ao cultivo bem-sucedido de *Macrolepiota bonaerensis* e *Tuber floridanum* (Maki & Paccola-Meirelles, 2002; Freiberg *et al*., 2023).

Objetivo geral

Investigar a diversidade, a viabilidade de cultivo e os aspectos nutricionais e etnomicológicos de cogumelos comestíveis silvestres da Mata Atlântica brasileira, com ênfase em espécies pertencentes aos gêneros *Macrolepiota*, *Macrocybe*, *Pseudohydnum* e *Tuber*.

Objetivos específicos

i. Descrever e delimitar espécies novas ou pouco conhecidas pertencentes aos gêneros *Macrolepiota*, *Macrocybe*, *Pseudohydnum* e *Tuber*, com base na integração de dados morfológicos e evidências moleculares;

ii. investigar as relações filogenéticas das linhagens brasileiras dos gêneros supracitados por meio de análises filogenéticas baseadas em dados moleculares multilocus, utilizando marcadores como ITS, LSU, *rpb1, rpb2* e *tef1-α*;

iii. descrever os usos tradicionais e o perfil nutricional de espécies selecionadas, considerando seu potencial alimentício e o conhecimento etnomicológico local;

iv. avaliar a viabilidade de cultivo de espécies silvestres promissoras dos gêneros-alvo por meio de ensaios experimentais em substratos regionais, com o intuito de testar sua adaptabilidade às condições de cultivo e subsidiar sua domesticação e potencial inserção na cadeia produtiva de cogumelos comestíveis no Brasil.

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Capítulo 1

Macrolepiota capelariae (Agaricaceae, Basidiomycota): a new species from the Brazilian Atlantic Rainforest with extended records to Argentina and Mexico

Artigo publicado no periódico **Phytotaxa**

Macrolepiota capelariae (Agaricaceae, Basidiomycota): a new species from the Brazilian Atlantic Rainforest with extended records to Argentina and Mexico[2]

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Abstract

A new *Macrolepiota* species from Brazil, *M. capelariae*, is proposed here based on morphological and molecular evidence. The description and illustrations are based on specimens collected in a remnant of the Atlantic Rainforest in São Paulo, Southeast Brazil. Analyses of nrITS sequences supported the recognition of this new species, in combination with morphological evidence, and revealed its phylogenetic placement in *Macrolepiota* sect. *Macrolepiota*. *Macrolepiota capelariae* is characterized by its medium to large and very tall basidiomata, light brown to pale greyish brown, brownish orange or clay brown pileus surface, ellipsoid to oblong, dextrinoid basidiospores with a distinct germ pore, narrowly clavate to sometimes clavate cheilocystidia, a trichodermal pileus covering composed of mostly clavate to narrowly clavate, thick-walled terminal elements, and absence of clamp connections. Nomenclatural types are deposited at the herbarium SP from the 'Instituto de Pesquisas Ambientais', São Paulo, Brazil. A comparison with allied species is provided. An earlier varietal name proposed based on a Brazilian material, viz. *Lepiota procera* var. *vulpina* Rick, could corresponds to a synonym of the new taxa, but the lack of nomenclatural type, extant original herbarium specimens, and the record of confident distinctive characters for this variety prevent a reliable correlation with the new species proposed.

Keywords: nrITS, phylogeny, integrative taxonomy, biodiversity, Neotropics

Introduction

Macrolepiota Singer (1948:141) was proposed to include members of Agaricaceae with white to cream color spore print, clamped hyphae, and giant basidiospores with a metachromatic inner wall in cresyl blue. Later, Heinemann (1989), Singer (1986), and Vellinga *et al.* (2003) complemented the recognition of the genus, characterizing it by including members with relatively large and

[2] Capítulo que originou o artigo "*Macrolepiota capelariae (Agaricaceae, Basidiomycota): a new species from the Brazilian Atlantic Rainforest with extended records to Argentina and Mexico*" publicado na Phytotaxa 22/12/2022 (https://doi.org/10.1590/1676-0611-BN-2024- 1701) – Anexo A.

fleshy basidiomata; a stipe with a complex, mobile ring and a striking, banded appearance due to the presence of hymeni-trichodermal patches; lamellae free to remote; with relatively large and ellipsoid to amygdaloid-ellipsoid basidiospores, which have a rounded apex with a germ pore covered by a hyaline cap; pleurocystidia absent; a trichodermal pileus covering; and clamp connections present or more rarely absent.

The traditional infrageneric classification adopted by Singer (1986) recognized two sections based on the presence or absence of clamp connections, viz. *Macrolepiota* sect. *Macrolepiota* with clamp connections on the hyphae of the trama or stipe or at the base of the basidia; and *Macrolepiota* sect. *Macrosporae* (Singer) Bon (1979:40) without clamp connections on the trama or present only at the base of cheilocystidia. Later, Ge *et al.* (2010), based on molecular analyses with nuc rITS1-5.8-ITS2 (nrITS) sequences, proposed a new section, viz. *Macrolepiota* sect. *Volvatae* Z.W. Ge, Zhu L. Yang & Vellinga (Ge *et al.* 2010:97), to accommodate representatives with volva on the stipe base, small and amygdaliform-ellipsoid basidiospores, and no clamp connections. The authors also recovered in their analyses the clades corresponding to *Macrolepiota* sect. *Macrolepiota* and *Macrolepiota* sect. *Macrosporae*.

Macrolepiota, with a widespread distribution, can be found in various habitats, from grasslands to native forests, and even in man-made habitats, such as gardens, lawns, and compost-heaps (Vellinga *et al.* 2003, Sysouphanthong *et al.* 2021). It appears to be geographically structured, with closely related species in the same area, and no known widely distributed species (Vellinga 2003, Vellinga *et al.* 2003, Ge *et al.* 2010, Vizzini *et al.* 2011, Cho *et al.* 2019). For instance, *M. procera* (Scop.) Singer (1948:141), an assumed widespread species, is now known to only occurs in Europe and temperate Asia. Its occurrence outside the Eurasian continental area has not been confirmed (Vellinga 2003, Vellinga *et al.* 2003, Ge *et al.* 2010).

While *Macrolepiota* species from the Northern Hemisphere are well-understood, the scarce diversity data for Neotropical *Macrolepiota* has helped to perpetuate taxonomic confusion regarding species delimitation and distribution. This is in part due to the widespread use of species epithets typified by specimens from Europe [viz. *M. procera*, *M. mastoidea* (Fr.) Singer (1951:417), *M. excoriata* (Schaeff.) Wasser (1978:516), and *M. fuligineosquarrosa* Malençon (1979:261)] for Neotropical collections, and the overlapping suites of morphological traits in the basidiomata of species in this genus (Vellinga *et al.* 2003). The recent inclusion of molecular approaches to taxonomic studies revealed the uncertainty of previous morphological identifications of Neotropical *Macrolepiota* species (Fazolino Perez *et al.* 2018, Freitas & Menolli 2019), and it now becomes clear that those species need to be re-examined using molecular data and described as independent unique taxa.

A total of 15 species of *Macrolepiota* has been reported from five states in Brazil, but the occurrence of species described from Europe, Africa, Asia, and Australia still has to be confirmed by molecular methods, and is in fact not likely:

i) *M. bonaerensis* (Speg.) Singer (1951:417) from the states of Minas Gerais (Rosa & Capelari 2009), Paraná (Meijer 2006, 2008, Maki & Paccola-Meirelles 2002), Rio Grande do Sul [Rick 1907, 1908 as *Lepiota bonaerensis* (Speg.) Speg. (Saccardo 1887:28), 1939 as *Lepiota excoriata* f. *bonaerensis* (Speg.) Rick (1939:318), 1961 as *L. bonaerensis*, Singer 1954 as '*Lepiota procera* f. *bonaerensis* (Speg.) Rick', Guerrero & Homrich 1983, Putzke *et al.* 2014], and São Paulo (Spegazzini 1889 as *L. bonaerensis*, Pegler 1997);

ii) *M. brasiliensis* (Rick) Raithelh. (Raithelhuber 1988:64) from Rio Grande do Sul [Rick 1907, 1939, 1961 as *Lepiota permixta* var. *brasiliensis* Rick (1907:68), Raithelhuber, 1988, 1991];

iii) *M. colombiana* Franco-Mol. (Franco-Molano 1999:14) from Rio Grande do Sul (Putzke *et al.* 2014) and Paraná (Ferreira & Cortez 2012);

iv) *M. cyanolamellata* Fazolino, Lechner & Suaza Blandoìn (Fazolino Perez *et al.* 2018:934) from Rio Grande do Sul (Fazolino Perez *et al.* 2018);

v) *M. dolichaula* (Berk. & Broome) Pegler & R.W. Rayner (1969:365) from São Paulo (Grandi *et al.* 1984);

vi) *M. dunensis* D.S. Freitas & Menolli (2019:228) from Rio Grande do Norte (Freitas & Menolli 2019);

vii) *M. excoriata* from Rio Grande do Sul [Rick 1907, 1939, 1961 as *Lepiota excoriata* (Schaeff.) P. Kumm. (Kummer 1871:135), Raithelhuber 1988, 1991];

viii) *M. fuligineosquarrosa* from Rio Grande do Sul (Alves *et al.* 2016);

ix) *M. kerandi* (Speg.) Singer (1951:417) from Rio Grande do Sul (Putzke *et al.* 2014);

x) *M. mastoidea* from Minas Gerais (Rosa & Capelari 2009), Rio Grande do Sul [Rick 1939, 1961 as *Lepiota procera* f. *gracilenta* (Krombh.) Rick (1939:318), Raithelhuber 1988, 1991 as *M. gracilenta* var. *acuteumbonato* Raithelh. (1988:64), Putzke *et al.* 2014, Alves *et al.* 2016 as *M. gracilenta* (Krombh.) Wasser (1978:516)], and São Paulo (Grandi *et al.* 1984);

xi) *M. procera* from Rio Grande do Sul [Rick 1939 as *Lepiota procera* var. *vulpina* Rick (1939:317), Alves *et al.* 2016] and São Paulo (Bononi *et al.* 1984);

xii) *M. pulchella* de Meijer & Vellinga (Vellinga & Yang 2003:184) from Paraná [Heinemann & de Meijer 1996 as *Volvolepiota brunnea* (Rick) Singer (1959:12), Vellinga & Yang 2003, Meijer 2006], Rio Grande do Sul [Rick 1938, 1961 both as *Lepiotella brunnea* Rick (1938:251); Singer 1954, 1959 both as *V. brunnea*], and São Paulo (Bononi *et al.* 1981 as *V.*

brunnea, Freitas & Menolli 2019);

xiii) *M. sabulosa* Fazolino & R.M. Silveira (Fazolino *et al.* 2018:936) from Rio Grande do Norte [Fazolino Perez *et al.* 2018, Freitas & Menolli 2019 as *M. sabulosa* var. *velistellaris* D.S. Freitas & Menolli (Freitas & Menolli 2019:234)];

xiv) *M. stercoraria* (Rick) Raithelh. (Raithelhuber 1988:63) from Rio Grande do Sul [Rick 1939, 1961 as *Lepiota stercoraria* Rick (1939:318), Raithelhuber 1988, 1991];

xv) *M. zeyheri* (Fr.) Singer (1962:67) from Rio Grande do Sul (Raithelhuber 1988, 1991).

Fazolino Perez *et al.* (2018) and Freitas & Menolli (2019) were the first authors to infer the phylogenetic positioning of Brazilian collections of *Macrolepiota* based on nrITS sequences of six species: *M. cyanolamellata* (Fazolino Perez *et al.* 2018), *M. dunensis* (Freitas & Menolli 2019), *M. sabulosa* (Fazolino Perez *et al.* 2018, including *M. sabulosa* var. *velistellaris* by Freitas & Menolli 2019), *M. pulchella* (Freitas & Menolli 2019, including the record of "*Macrolepiota* sp. 3" by Fazolino Perez *et al.* 2018), and the unnamed *Macrolepiota* sp. 1 and sp. 2 (Fazolino Perez *et al.* 2018).

Here, a new species of *Macrolepiota* is described from a fragment of the Atlantic Rainforest in São Paulo, Southeast Brazil. The new species was compared to previously described *Macrolepiota* species from the world literature, and their novelty demonstrated by comparison with morphologically similar described species and by molecular data (nrITS).

Materials and methods

Collection site

The collections of the new taxa have been found for many years in the 'Parque Estadual das Fontes do Ipiranga' (PEFI), a State Park that represents a remnant of the Atlantic Rainforest in São Paulo, Southeast Brazil. The forest is mainly composed of plant members of Myrtaceae, Fabaceae, Lauraceae, Melastomataceae, and typical genera such as *eugenia*, *Myrcia*, *Ocotea*, and *Psychotria* (Barros *et al.* 2002). The PEFI is a large fragment of Atlantic Rainforest (526 ha) inserted in the urban area of the metropolitan region of São Paulo city, state of São Paulo, between 23º 38' 08" S–23º 40' 18" S and 46º 36' 48" W–46º 38' 00" W (Fernandes *et al.* 2002). The Park is at elevations between 770 and 825 m a.s.l (Nastri *et al.* 1992), with its predominant vegetation classified as Dense Ombrophilous Forest (Veloso *et al.* 1991), and a temperate climate with warm summers and dry winters (Cwa), according to the Köppen-Geiger climate classification system (Kottek *et al.* 2006, Peel *et al.* 2007).

Data from the Global Biodiversity Information Facility (GBIF), which covers data from iNaturalist (https://www. inaturalist.org.), and the Mushroom Observer (https://mushroomobserver.org) were consulted to access the geographical occurrence of

specimens macromorphologically similar to the new species and which putatively represent the same taxon. Photos from the aforementioned platforms were downloaded to illustrate the species macromorphological identity from records outside the type locality. We followed the Creative Commons (https://creativecommons.org/) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CC BY, CC BY-NC, CC BY-SA, CC BY-ND, CC BY-NC-ND, and CC BY-NC-SA; for licenses and code attributions, please check https://creativecommons.org/licenses/?lang=en).

This study is according to the Brazilian legislation on access to biodiversity and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen #ABA9DB4).

Morphological methods, notation and use of standards

Macromorphological descriptions were based on field notes and color photographs of basidiomata taken in the field. Basidiomata were dried in a hot air dehydrator (45 °C). Color notation of fresh basidiomata is according to Küppers (2002), with color codes noted in parentheses (e.g., $N_{60}C_{60}Y_{50}$). Details of the basidiomata surface were examined under a Zeiss Stemi 305 stereo microscope.

Microscopic analyses were performed by a Zeiss Axioscope 5 microscope with bright field, phasecontrast optics and a charge-coupled device camera (Zeiss Axiocam 208 color), using sections of fresh or rehydrated tissues. Dried samples were rehydrated in distilled water or moistened in ethanol 70%, and microsections were cut by hand with a razor blade. Microscopic structures were examined in water, 3% KOH, Congo red, Cotton Blue, Cresyl Blue and Melzer's reagent, separately. At the beginning of a set of basidiospore data, the abbreviation [*a/b/c*] signifies "*a*" basidiospores measured from "*b*" basidiomata of "*c*" collections. Dimensions of basidiospores are presented in the following form *(m–) n–o (–p),* in which "*m*" is the smallest value observed or calculated and "*p*" is the largest value observed or calculated. In the range of values observed or calculated, the 5th percentile is "*n*"; and the 95th percentile is "*o*". A summary of definitions of biometric variables follows:

$\mathbf{L_m}$, ($\mathbf{W_m}$) = the average of all lengths (widths) of basidiospores measured.

Q = the ratio of length to width of a basidiospore or the range of such ratios for all basidiospores measured.

$\mathbf{Q_m}$ = the average of all Q values computed for all basidiospores measured.

The form of the basidiospores was interpreted based on the Q values following Bas (1969), and descriptive terms for other morphological features follow Vellinga (1988) and Vellinga & Noordeloos (2001). The width of the basidia was taken at the widest part and the length was measured from the apex without sterigmata to the basal septum.

The collections examined including the selected nomenclatural types are deposited at the mycological collection of the herbarium SP from the 'Instituto de Pesquisas Ambientais', São Paulo, Brazil (Thiers, continuously updated).

Molecular methods and phylogenetic analyses

The procedure of extraction and amplification of DNA was based on the protocol of Binder & Hibbett (2003) from small parts of lyophilized material or directly from dried basidiomata fragments. The nrITS region was amplified using the primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993). Sequences of the nuclear large subunit (nLSU) were generated for the holotype (*DAZ060*) and two paratypes collections (*MC4584*, *MC4588*) using the primers LROR and LR5 (Vilgalys & Hester 1990, James *et al.* 2006), although they were not used in the phylogenetic analyses. The PCR products were cleaned with PureLink PCR Purification Kit (Invitrogen) according to the manufacturer's instructions and then sequenced using the same primer pairs.

Phylogenetic analyses were conducted with *Leucoagaricus leucothites* (Vittad.) Wasser (1977:308) and *Leucoagaricus nympharum* (Kalchbr.) Bon (1977:19) as outgroup and including sequences of *Macrolepiota* from GenBank based on sampling for recent phylogenetic analyses of Brazilian collections (Fazolino Perez *et al.* 2018, Freitas & Menolli 2019) with the additional sequences generated here (ON753529–ON753534). GenBank accession numbers for each collection are given in Fig. 1. The alignment was conducted using MAFFT 7.222 (Katoh & Standley 2013) with auto strategy and edited manually in Geneious 7.0.6 (Kearse *et al.*, 2012). The best nucleotide evolution model was estimated using the AIC criterion (Akaike Information Criterion) in PartitionFinder 2.1.1 (Lanfear *et al.* 2012, 2016, Guindon *et al.* 2010). The nrITS region was partitioned into ITS1, 5.8S and ITS2, and the evolution model was estimated for each partition. A Maximum Likelihood (ML) analysis was performed in RAxML 8.2.12 (Stamatakis 2014) in the CIPRES Science Gateway 3.3 (Miller *et al.* 2010) using the GTR+GAMMA model with a rapid bootstrap analysis with 1,000 replicates and search for the best-scoring ML tree. The Bayesian inference (BI) analysis was performed with MrBayes v3.2.7a (Ronquist *et al.* 2012) in the CIPRES Science Gateway 3.3 (Miller *et al.* 2010) using three partitions (ITS1, 5.8S, ITS2), with TVM+G model for ITS1 and ITS2, and JC model for

5.8S, with two independent runs, four simultaneous independent chains and 10,000,000 generations with a sample frequency every 5,000 generation. The following abbreviations are used for statistical data: Bootstrap (BS) and Posterior Probability (PP).

Results

Phylogeny

The nrITS amplification products of the analyzed samples ranged from 400–644 bp. A total of 94 sequences were used for the nrITS phylogeny, including the six newly sequenced collections and the outgroups. The multiple sequence alignment was 723 characters long in total.

Both ML and BI analyses resulted in similar tree topologies and so only the ML tree is illustrated (Fig. 1) with both support values shown near the clade nodes. It was verified, with the highest support (100% BS, 1 PP), the monophyly of the genus, which is organized in three main clades corresponding to the sections indicated by Ge *et al.* (2010): *Macrolepiota* sect. *Macrolepiota* (unsupported), *Macrolepiota* sect. *Macrosporae* (89% BS, 1 PP), and *Macrolepiota* sect. *Volvatae* (100% BS, 1 PP).

All sequences generated here from the new species formed a well-supported clade (97% BS, 1 PP) within the clade corresponding to *Macrolepiota* sect. *Macrolepiota* and together with two sequences (MN847716 and MH290361) of two unnamed collections from Argentina and Mexico that most likely also represent *M. capelariae*. All sequences in *M. capelariae* clade are 98.78–100% identical, with MN847716 (Argentina) and MH290361 (Mexico) presenting, respectively, levels of 0.31% and 0.93% sequence divergence compared to the holotype of *M. capelariae*. *Macrolepiota capelariae* is related (86% BS, 1 PP) to three other *Macrolepiota* species, viz. *M. colombiana* from Colombia and two clades corresponding to species of unnamed collections from Argentina and Brazil (Fig. 1).

Taxonomy

Macrolepiota capelariae A.D. Souza, C.C. Nascimento & Menolli *sp. nov.* (Figs. 2–4)
MycoBank: MB 844529

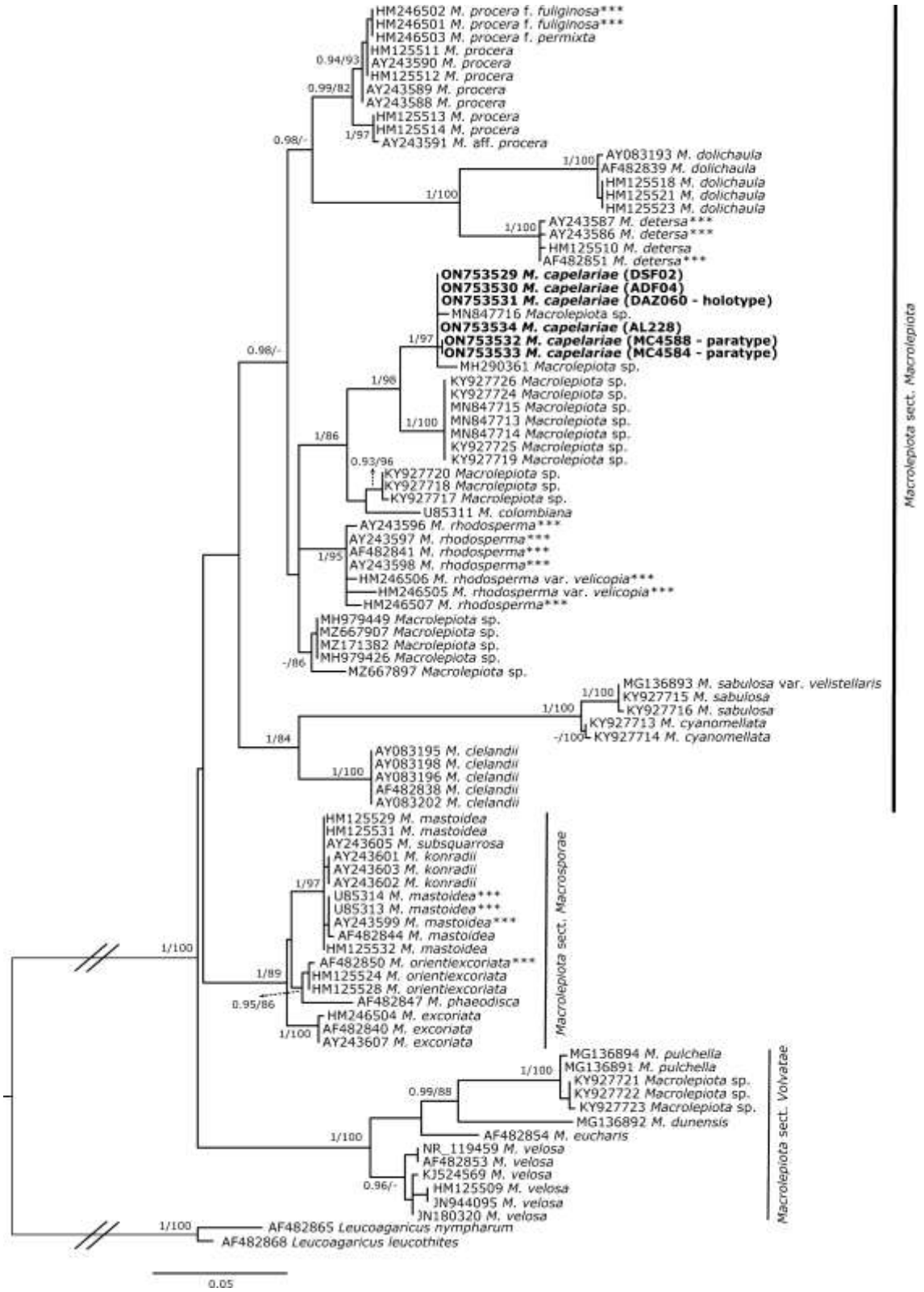

FIGURE 1. Best tree from the ML analysis of *Macrolepiota* based on nrITS data and rooted with *Leucoagaricus leucothites* and *L. nympharum*. The sequences generated in this work are in bold. Support values (BS >70% and PP >0.90) are shown near the node branches. Root length has been reduced to facilitate graphical representation. A dash (-) indicates that the support values for the clade are below those indicated above. *** indicates taxon names modified according to Vizzini *et al.* (2011).

Diagnosis:—Similar to *Macrolepiota colombiana* but differing in brownish orange or clay brown, light brown to pale greyish brown pileus surface, mostly breaking up into radially

arranged interwoven strips, and then exposing a whitish background, the less complex annulus, the unchanging context, the non-septate cheilocystidia, and absence of clamp connections.

Etymology:—'*capelariae*', in honor of Dr. Marina Capelari, a dedicated Brazilian mycologist for her contribution to the taxonomic study of mushrooms from Brazil for more than 30 years during her career.

FIGURE 2. *Macrolepiota capelariae* (SP513052, holotype). **A.** Young basidiomata. **B.** Mature basidioma. **C.** Detail of the lamellae. **D.** Detail of the annulus. **E.** Detail of the stipe base. Scale bars = 20 mm. Photos by D.A. Zabin.

FIGURE 3. Putative records of *Macrolepiota capelariae* outside the type locality. Data from GBIF/iNaturalist and Mushroom Observer. **A**–**K.** Brazil (**A**, **J**, **K**: Rio de Janeiro, RJ; **B**: Rio Branco, AC; **C**: Lajeado, RS; **D**: Jaraguá do Sul, SC; **E**: Florianópolis, SC; **F**: Palmeira, PR; **G**: São Sebastião da Grama, SP; **H**: Vitorino, PR; **I**: Senador Guiomard, AC). **L.** Mexico (Tepoztlán, Morelos). **Brazilian states abbreviations**: AC = Acre, PR = Paraná, RJ = Rio de Janeiro, RS = Rio Grande do Sul, SC = Santa Catarina, SP = São Paulo. **Photo credits**: **A.** ©Rogerio Dias, **B.** ©Gabriel Fernando, **C.** ©Edith Mello, **D.** ©fernandotorres1978, **E.** ©Mariana Lopes, **F.** ©andersonwarkentin, **G.** ©Café Gato-Mourisco, **H.** ©Alan Lucas Hentz Policarpo, **I.** ©Mayk Oliveira, **J.** ©rogerriodias, **K.** ©Alessandro Valente Vieira, **L.** ©Alejandro Tux.

Holotype:—BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 14 January 2022, *D.A. Zabin & M.C.S. Pires DAZ060* (SP513052), GenBank [nrITS]: ON753531, [nLSU]: ON794497.

Paratypes:—BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 24 July 2010, *M. Capelari, P.O. Ventura & N. Menolli Jr. MC4584* (SP513053), GenBank [nrITS]: ON753533, [nLSU]: OP302841; *ibid.* 06 December 2010, *M. Capelari MC4588* (SP513054), GenBank [nrITS]: ON753532, [nLSU]: OP302842; *ibid.* 20 March 2015, *D.S. Freitas DSF02* (SP513055), GenBank [nrITS]: ON753529; *ibid.* 800 m a.s.l, 26 February 2016, *A.D. Souza & L.S. Ramos ADF04* (SP513056), GenBank [nrITS]: ON753530; *ibid.* 03 March 2016, *A.A. Alcântara AL228* (SP513057), GenBank [nrITS]: ON753534.

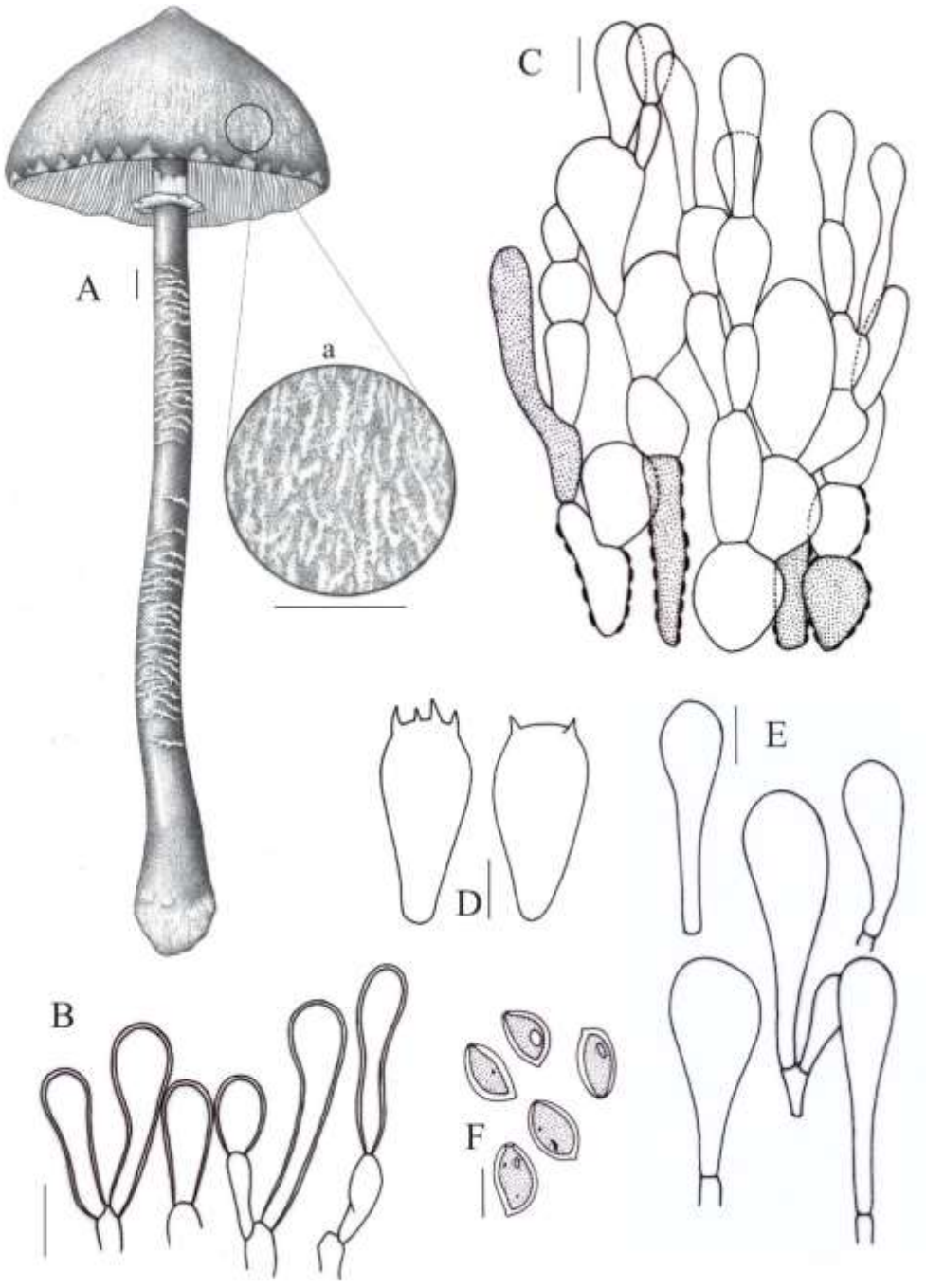

FIGURE 4. *Macrolepiota capelariae* (SP513052, holotype). **A**. Mature basidioma, **a**. Detail of the pileus surface. **B**. Apical thick-walled elements of the pileus covering. **C**. Pileus covering. **D**. Basidia. **E**. Cheilocystidia. **F.** Basidiospores. Scale bars: A/a = 10 mm; B–F = 10 µm. Drawings: A/a by K. Sousa, B–F original by C.C. Nascimento and inked by K. Sousa.

Description:—*Basidiomata* medium to large-sized. *Pileus* 78–120 mm diam., fleshy, ovoid to hemispherical when young, expanding to broadly campanulate, convex, rounded-umbonate, obtuse umbonate to plano-umbonate, sometimes applanate with a slightly reflexed margin with age; surface dry, at first brownish yellow ($N_{20}Y_{70\text{-}90}M_{30}$) to brownish orange ($N_{20}Y_{70\text{-}90}M_{30}$), then light brown ($Y_{30\text{-}40}M_{30}C_{20}$, $Y_{40}M_{30\text{-}40}C_{30}$) to pale greyish brown ($N_{40}Y_{30\text{-}50}M_{20}$), brownish orange ($Y_{60}M_{30\text{-}40}C_{10\text{-}20}$) or clay brown ($Y_{40}M_{50}C_{20}$), breaking up into radially arranged interwoven strips, but sometimes into small to large patch-like squamules (easily detachable from the pileus), and then exposing a whitish background; disc smooth, dark brown ($N_{80}A_{90}M_{50}$–$N_{90}A_{20}M_{60}$) to reddish brown ($N_{50}Y_{80}M_{50}$–$N_{60}Y_{80}M_{70}$). *Lamellae* free, remote from the stipe, crowded, ventricose, white when young, white to beige ($Y_{20}M_{10}C_{10}$) when mature, sometimes with brownish spots; lamellulae attenuate, in 1–2 ranks, unevenly distributed; edge entire, smooth, concolorous. *Stipe* 150–320 × 5–18 mm, central, subcylindrical, gradually attenuating upwards, bulbous at base; bulb subglobose, 20–25 mm wide, completely covered by a tomentose-velvety whitish mycelial layer, often with long white rhizomorphs; surface medium brown ($N_{50}Y_{60}M_{30}$–$N_{60}Y_{40\text{-}50}M_{40}$), paler upwards, finely fibrillose, sometimes breaking open into pale brown ($Y_{60}M_{50}C_{50}$) zigzagging bands over the middle zone on an off-white background. *Annulus* superior, membranous, beige ($N_{40}A_{10}M_{10}$) at the upper side, pale brown at underside, with a broken brownish margin, movable when mature. *Context* in the pileus white and moderately thick, in stipe white and hollow with a central white cottony strand, unchanging in both. *Odor* fungoid. *taste* mild, sweet. *Spore print* white.

Basidiospores [255/16/16] (8.8–)12.5–15.0(–17.5) × (5.8–)7.5–11.2(–11.8) µm ($\mathbf{L_m}$= 13 µm; $\mathbf{W_m}$ = 8.5 µm; Q = 1.3–2.0; $\mathbf{Q_m}$ = 1.54), ellipsoid to elongate, with germ pore covered by hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, metachromatic in Cresyl blue; apiculus about 1 µm long. *Basidia* 22–42 × 11.2–15.0(–16.2) µm, clavate, hyaline, thin-walled, 1–4-spored. *Lamella edge* sterile, with crowded cheilocystidia. *Cheilocystidia* 21–50 × 9.0–15.0(–16.5) µm, narrowly clavate, sometimes clavate, often catenulate, hyaline, colorless, thin-walled. *Pleurocystidia* absent. *Pileus covering* a trichoderm composed of 4–6 layers of elements, often branching; lower layer elements subglobose, broadly clavate to oblong, seldom cylindrical, 18.0–32 × 11.0–25 µm, slightly thick-walled to thick-walled, with pale yellowish brown intracellular pigments, walls sometimes encrusted; upper

layer elements mostly clavate to narrowly clavate, 11.5–32 × 3.0–11 µm, slightly thick-walled to thick-walled, with hyaline to pale yellowish brown parietal pigment. *Stipe covering* a hymeniderm of cylindrical elements, 6.2–14.7 µm wide, thin- walled, with intracellular pale brown content. *Clamp connections* absent.

Habitat and distribution:—Saprotrophic and terrestrial, growing in a remnant of Atlantic Rainforest, under shade trees, solitary or in small groups, known from the state of São Paulo, Southeastern Brazil (type locality). Based on records from GBIF, Mushroom Observer, and GenBank (MN847716 and MH290361), the species distribution can be extended to Argentina and Mexico, and putatively to five other Brazilian states: Acre, Paraná, Rio de Janeiro, Rio Grande do Sul, and Santa Catarina.

Additional specimens examined:—Brazil, SP, São Paulo, Parque Estadual das Fontes do Ipiranga (PEFI), 800 m a.s.l., 14 October 1998*, L. Gusmão s.n.* (SP513059); *ibid.* 15 October 1998*, M.Capelari s.n.* (SP513060); *ibid.* 10 October 2001, *U.C. Peixoto s.n.* (SP381602); *ibid.* 26 December 2001, *U.C. Peixoto s.n.* (SP381601); *ibid.* 05 September 2002*, U.C. Peixoto s.n.* (SP381603); *ibid.* 11 December 2003, *M.D.F. Trude LJG 022/03* (SP381600); *ibid.* 30 January 2008*, T.V.S. Campacci s.n.* (SP513061); *ibid.* 04 April 2012, *M. Capelari MC4692* (SP513062); *ibid.* 26 February 2016, *A.D. Souza ADF03* (SP513058).

Discussion

Among the taxa in *Macrolepiota* sect. *Macrolepiota* not sampled in the phylogenetic tree, the following must be considered to support the morphological differences and the proposition of novelty for *M. capelariae*: (1) *Lepiota procera* var. *vulpina*, (2) *M. bonaerensis*, (3) *M. africana* (R. Heim) Heinem. (Heinemann 1969:207), (4) *M. zeyheri*,
(5) *M. brasiliensis*, and (6) *M. stercoraria*. Additionally, *M. colombiana*, which is sampled in our phylogenetic analyses based on authentic material from Colombia (Franco-Molano, 1999), is also compared to *M. capelariae*, although our nrITS tree (Fig. 1) clearly supports the molecular distinction of both species. Furthermore, based on the pictures, collecting area and/or morphological descriptions presented by Bononi *et al.* (1984), Grandi *et al.* (1984), Ferreira and Cortez (2012), and Alves *et al.* (2016), the records of *M. procera*, *M. colombiana*, and *M. mastoidea* by these authors most likely also represent *M. capelariae*, although a careful re-examination of the samples studied by them is necessary to confirm this.

Lepiota procera var. *vulpina* comes quite close to *M. capelariae*. However, it was described by Rick (1939) without any holotype indication and based on a pretty short Latin protologue: “colore vulpine-fulvo et squamis innatis. Color Lep. procerae generatium est griseo-

brunneus". Although the color and ornamentation of the pileus as described by Rick (1939) resemble those of *M. capelariae*, the lack of extant original herbarium specimens and record of any other distinctive character for *L. procera* var. *vulpina* prevent a reliable correlation with the new species proposed here. Nevertheless, the characters described by Rick (1939) for a supposed typical variety of *L. procera* (= *M. procera*) from Brazil are distinct from those recorded to *M. capelariae*, such as the larger pileus (up to 30 cm wide) and the longer basidiospores (14–22 µm). Rick (1961) also listed *Lepiota gigantea* Speg. (Spegazzini 1909:260) as a synonym of *L. procera* var. *vulpina*, but still the first's original description does not resemble *M. capelariae* in some recorded characters. *Lepiota gigantea*, known from Argentina, differs from *M. capelariae* in its much larger size, with a maximum reported pileus diameter of 25 cm and stipe length/width of 90 × 3 cm, and by a distinctly acute umbonate pileus with a striate margin; no data is provided for the basidiospores (Spegazzini 1909). A study with a thorough taxonomic review of the *Macrolepiota* species described from the Neotropics, and all relevant Rick's names, could help to verify if *Lepiota procera* var. *vulpina* should be listed as synonym of *M. capelariae*, but no collection under this varietal name were located at PACA herbarium (curator pers. comm.) neither at the database (https://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm) of the 'Lloyd Herbarium' collections that are now part of the U.S. National Fungus Collections (BPI), both where the Rick's collections are deposited.

Macrolepiota bonaerensis, described as *Agaricus bonaerensis* Speg. (Spegazzini 1880:278) from Argentina and then combined in *Macrolepiota* by Singer (1951:417), differs from *M. capelariae* in its smaller basidiomata (pileus 50–90 mm diam., stipe 80–10 × 5–10 mm), conspicuous whitish pileus background, fimbriate to appendiculate pileus margin, larger basidiospores (14.5–16 × 11.3–11.8 µm), and clamped hyphae in the stipe context (Spegazzini 1880, Singer 1951, Singer & Digilio 1952). *Macrolepiota bonaerensis* was reported from grassy habitats, always outside woodland, and in a totally different habitat than that of *M. capelariae* (Spegazzini 1880, Singer 1951, Singer & Digilio 1952).

When compared to *M. capelariae*, *M. africana*, originally described from Central African Republic and Cameroon (Heim 1968:212 as *Leucocoprinus africanus*) but later reported from other parts of Africa (Heinemann 1969, Pegler 1977, Härkönen *et al.* 2003), has a larger pileus [100–150(–300) mm] with radially tomentose dark brown surface, a more complex annulus with brown squamules on the lower surface, slightly smaller basidiospores [12.2–13.9(–15) × 7.6–9.9 µm], and clamped hyphae in the context (Heinemann 1969, Pegler 1977). Moreover, the trichodermal layer of

M. africana is composed of cylindrical, septate hyphae that seldom branch, with some terminal elements (40–120 × 6– 13 µm) developing a thick brown wall and an acutely pointed apex (Heinemann 1969; Pegler 1977). In *M. capelariae*, the trichodermal layer is formed of slightly thick-walled to thick-walled terminal elements with an obtusely rounded apex, not exceeding 32 µm long.

Macrolepiota zeyheri, originally described from South Africa as *Agaricus zeyheri* Fr. (Fries 1848:122), can be distinguished from *M. capelariae* by the appendiculate pileus margin, the whitish cream to pinkish lamellae, stipe length [60–80(–100) mm] that rarely exceeds the pileal diameter (50–100 mm), and slightly smaller basidiospores,
11.5–15 × 7.5–10.5 µm (Pearson 1950, Heinemann 1969, Reid 1975, Pegler 1982, Raithelhuber 1988, 1991).

Macrolepiota brasiliensis and *M. stercoraria* were originally described from Southern Brazil as *Lepiota permixta* var. *brasiliensis* (Rick 1907:68) and *L. stercoraria* (Rick 1939:318), respectively, although with very short protologues. *Macrolepiota stercoraria* was described in more detail by Raithelhuber (1988). *Macrolepiota brasiliensis* and *M. stercoraria* are both of considerably smaller stature than *M. capelariae*, with a maximum reported stipe length of 120 mm, which does not reach the smallest stipe size observed for *M. capelariae*. In addition, *M. brasiliensis* differs from
M. capelariae in having a whitish pileus, pale green tinge on lamellae, and smaller basidiospores (9.5–12.2 × 6–7 µm); whilst *M. stercoraria* is distinguished from *M. capelariae* in its brownish to ochraceous pileus squamules that are concentrically arranged on pileus surface, a depressed pileus center, and a yellowish annulus (Rick 1907, 1939, 1961, Raithelhuber 1988, 1991). Additionally, whether these taxa indeed belong to *Macrolepiota* and not to *Chlorophyllum* Masse (1898:135) still needs to be shown.

Finally, *M. colombiana*, originally described from Colombia in *Quercus humboldtii* Bonpl. (Humboldt & Bonpland 1809:155) and Pinaceae forests, is also characterized by robust basidiomata but differs from *M. capelariae* in phylogenetic placement (Fig. 1) and its dark brown central calotte at mature pileus that has large to small scales on a white background, more complex annulus, mostly septate and often branched cheilocystidia, and abundant clamp connections on stipe hyphae (Franco-Molano, 1999). Furthermore, *M. colombiana* has a white context slowly turning grayish-red when exposed and a very strong odor of cabbage (Franco-Molano, 1999).

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Capítulo 2

Documenting the diversity, edibility, and nutritional value of *Macrolepiota* mushrooms from Brazil with the proposal of three new edible species based on morphotaxonomy, multigene phylogenetic analyses and ethnomycological evidence

Artigo a ser submetido ao periódico **Persoonia**

Documenting the diversity, edibility, and nutritional value of *Macrolepiota* mushrooms from Brazil with the proposal of three new edible species based on morphotaxonomy, multigene phylogenetic analyses and ethnomycological evidence

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ABSTRACT

The lepiotaceous genus *Macrolepiota* Singer has been classified in the euagaric family Agaricaceae and comprises many edible species that are highly appreciated for their nutritional and culinary values. In recent years, molecular sequence analyses combined with ecological and morphological data have been used for species differentiation within the genus *Macrolepiota* from Brazil. Nevertheless, modern collections and molecular data are lacking for many species that are commonly wild harvested and consumed by some local citizens. During our ongoing research on edible mushrooms, several specimens of *Macrolepiota* were collected in Brazil and Paraguay by the senior author and collaborators. The sampled collections were identified based on morphological features and molecular phylogenetic inference from the analyses of four loci (ITS, nrLSU, *rpb2*, and *tef1-α*). In addition, to identify ethnomycologically important wild *Macrolepiota* species, data on their edibility were collected using semi-structured interviews, focus group discussions, participant observations, and walk-in-the-woods methods with selected key and general informants from communities in the Southern and Southeastern Brazil. Our results supported the introduction of three novel species and two novel varieties: *M. abruptibulbosa*, *M. chapeletae*, *M. pernuda*, *M. capelariae* var. *velana*, and *M. pulchella* var. *gymnopodia*. In addition, supplementary information on morphology, phylogenetic position, and distribution is presented to *M. bonaerensis*, *M. cyanolamellata*, and *M. sabulosa*. Three major clades within *Macrolepiota* were recovered on the phylogenetic analyses, which correspond to the traditional sections *Macrolepiota* sect. *Macrolepiota*, *Macrolepiota* sect. *Macrosporae*, and *Macrolepiota* sect. *Volvatae*. The edibility of *M.*

abruptibulbosa, *M. pernuda*, *M. pulchella*, *M. capelariae*, and *M. chapeleta* is documented for the first to science. The latter two species plus *M. bonaerensis* were also found to be sources of household income. Finally, the nutritional values of *M. bonaerensis*, *M. capelariae*, and *M. chapeleta* mushrooms were determined through proximate and mineral element analyses. Based on this assessment, it can be concluded that these species can serve as a valuable food source that can be exploited to meet the increasing food demands and reduce food insecurity in vulnerable communities in Brazil and nearby countries.

Keywords: Agaricaceae, lepiotaceous, ethnomycology**,** phylogeny, taxonomy, systematics

INTRODUCTION

The lepiotaceous genus *Macrolepiota* Singer is widely distributed (Vellinga 2004) and can be found in various habitats, from grasslands to native forests and even in man-made habitats, such as gardens, lawns, and compost-heaps (Vellinga *et al*. 2003, Sysouphanthong *et al*. 2021). It has been classified in the euagaric family Agaricaceae Chevall. of the Agaricoid clade (Matheny *et al*. 2006) and comprises some of the most conspicuous and beautiful mushrooms in the world, consisting of many edible species [e.g., *M. mastoidea* (Fr.) Singer, *M. detersa* Z.W. Ge, *M. dolichaula* (Berk. & Broome) Pegler & R.W. Rayner, and *M. procera* (Scop.) Singer] that are highly appreciated for their culinary values (Ge *et al*. 2010, Kumari & Atri 2014, Rizal *et al*. 2016, Li *et al*. 2020). The basidiomata of some *Macrolepiota* species (viz., *M. procera*, *M. mastoidea*, and *M. dolichaula*) also satisfy the criteria for a health-promoting food by their high protein content, rich mineral composition, low fat content, and for the presence of numerous bioactive substances with a very wide range of beneficial effects (Rizal *et al*. 2015, Kumari and Atri 2014, Ćirić *et al*. 2019, Erbiai *et al*. 2021, Adamska and Tokarczyk 2022). In addition, the genus plays important role in nutrient retention and recycling in tropical, subtropical, and temperate regions (Guzmán and Guzmán-Dávalos 1992, Rizal et al. 2015).

Macrolepiota was proposed to include members of Agaricaceae with white to cream color spore print, clamped hyphae, and giant basidiospores with a metachromatic inner wall in Cresyl blue (Singer 1948). Later, Heinemann (1989), Singer (1986), and Vellinga *et al.* (2003) expanded upon the recognition of the genus, which has been characterized by including members with relatively large and fleshy basidiomata; a stipe with a complex, mobile ring and a striking, banded appearance due to the presence of hymeni-trichodermal patches; free to

remote lamellae; relatively large and ellipsoid to amygdaloid-ellipsoid basidiospores, which have a rounded apex with a germ pore covered by a hyaline cap; pleurocystidia absent; a trichodermal pileus covering; and clamp connections present or more rarely absent.

Currently, ca. 40 *Macrolepiota* species have been described worldwide (Kirk *et al*. 2008, Ge *et al*. 2010, 2012, Fazolino Perez *et al*. 2018, Hyde *et al.* 2024), with more than 130 names listed in Index Fungorum (http://www.indexfungorum.org), including primarily agaricoid members with few secotioid taxa (Label *et al*. 2012).

Three sections have been recognized in *Macrolepiota* [viz. *Macrolepiota* sect. *Macrolepiota* (Singer 1948), *Macrolepiota* sect. *Macrosporae* (Singer) Bon (Bon 1979), and *Macrolepiota* sect. *Volvatae* Z.W. Ge, Zhu L. Yang & Vellinga (Ge *et al*. 2010)]. Members of *Macrolepiota* sect. *Macrolepiota* are characterized by having a complex annulus, relatively big ovoid-ellipsoid basidiospores, and a common presence of clamp connections at the base of the cheilocystidia and basidia (Bon 1996, Ge *et al*. 2010). Species of *Macrolepiota* sect. *Macrosporae* give rise to a simple annulus, smooth stipe, and rare clamp connections (Ge *et al*. 2010) Finally, the species of *Macrolepiota* sect. *Volvatae* have a volva at the stipe base, relatively small amygdaliform-ellipsoid basidiospores, and no clamp connections at the base of the cheilocystidia and basidia (Ge *et al*. 2010).

In recent years, molecular sequence analyses combined with ecological and morphological data have been used for species differentiation of *Macrolepiota* species from Brazil (Fazolino Perez *et al*. 2018, Freitas & Menolli 2019, Souza *et al.* 2022). This approach led to significant progress in the systematics of *Macrolepiota* with description of four new species (viz., *M. capelariae* A.D. Souza, C.C. Nascimento & Menolli, *M. cyanolamellata* Fazolino, Lechner & Suaza Blandoìn, *M. dunensis* D.S. Freitas & Menolli, and *M. sabulosa* Fazolino & R.M. Silveira) and the inference of the phylogenetic positioning of *M. pulchella* de Meijer & Vellinga (Fazolino Perez *et al*. 2018, Freitas & Menolli 2019, Souza *et al.* 2022).

Nevertheless, modern collections and molecular data are lacking from many species commonly harvested wild and consumed by some local citizens. These collections have been superficially identified by names typified by specimens from Europe or by the widespread South American names such as *M. bonaerensis* (Speg.) Singer and *M. colombiana* Franco-Mol. The putative occurrence of these two species in the Brazilian territory have not yet been confirmed by molecular data, and, therefore, the indiscriminate assignment of these names may be masking the recognition of some close undescribed species. A recent comprehensive study that aimed to access the diversity of edible mushrooms from Brazil based on literature data compiled the records of seven species of *Macrolepiota*: *M. bonaerensis*, *M. colombiana*, *M. dolichaula*, *M. excoriata* (Schaeff.) Wasser, *M. kerandi* (Speg.) Singer, *M. mastoidea*, *M.*

procera, and *M. zeyheri* Heinem. (Drewinski *et al*., 2024). These species had their edibility status previously categorized by Li *et al*. (2020) and mostly representing names originally described for Eurasia. From this perspective, integrative studies involving taxonomy and ethnomycology are still mandatory to clarify which edible *Macrolepiota* species actually occur in Brazil.

The nutritional value of non-Eurasian *Macrolepiota* species also remains largely unstudied. For example, the two recently described edible species from North America, namely *M. macilenta* Lebeuf, S.D. Russell & Justo and *M. pallida* Lebeuf, S.D. Russell & Justo (Lebeuf *et al*. (2024), as well as the edible species *M. bonaerensis* and *M. colombiana* from South America (Singer 1951, Franco-Molano 1999), lack information regarding their nutritional value and potential use as functional foods.

In order to bring the diversity, ethnomycological knowledge, and nutritional value of *Macrolepiota* from Brazil into a sharper focus, this study has the following specific goals: i) to describe three new species and two new varieties with presentation of complete morphological profiles allied to multilocus phylogenetic analyses; ii) to re-evaluate the systematic framework and support of the known sections within the genus; iii) to provide a dichotomous key to the *Macrolepiota* species from the Neotropics; and iv) to document the edibility of wild species in rural and semi-rural communities from Southeastern and Southern Brazil.

MATERIALS AND METHODS

Collection sites and photos copyright permissions

A total of 38 samples of *Macrolepiota* were collected by the senior author and collaborators from December 2019 to May 2024 in Northeastern, Midwestern, Southeastern, and Southern Brazil. The collection trips were conducted mainly during the rainy season, from December to April, in the states of Minas Gerais (MG), Mato Grosso do Sul (MS), Rio de Janeiro, Rio Grande do Norte (RN), Rio Grande do Sul (RS), and São Paulo (SP), which comprise areas of the following biomes: Atlantic Rainforest (MG, RJ, RN, RS, SP), 'Pantanal' (MS), and 'Pampa' (RS). In addition, 27 collections were sourced from inventories in Paraguay and on loans from the herbaria SP [Herbarium of the 'Instituto de Pesquisas Ambientais (IPA)'] and FLOR [Fungarium of the 'Universidade Federal de Santa Catarina (UFSC)'], with other seven samples analyzed *in loco* at the herbarium ICN of the 'Universidade Federal do Rio

Grande do Sul' (UFRGS) and at the fungarium UFRN-Fungos of the 'Universidade Federal do Rio Grande do Norte'.

Data from the Global Biodiversity Information Facility (GBIF), which covers data from iNaturalist (https://www.inaturalist.org.https://mushroomobserver.org), were consulted to access the type locality occurrence of specimens macromorphologically similar to *M. colombiana*. Photos from the aforementioned platforms were downloaded to illustrate *M. colombiana* macromorphological identity and its putative distribution. We followed the Creative Commons (https://creativecommons.org/) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CCBY, CC-BY-NC, CCBY-SA, CC-BY-ND, CC-BY-NC-ND, and CC-BY-NCSA; for licenses and code attributions, please check https://creativecommons.org/licenses/?lang=en).

This study is according to the Brazilian legislation on access to biodiversity and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen # 0000000)[3].

Morphological methods, notation and use of standards

To assess the macromorphological features, images of the basidiomata were taken using a Canon EOS 6D Mark II camera (Canon Inc., Tokyo, Japan) fitted with a 35 mm lens. Detailed observations on the size, color, odor, and taste of the basidiomata were made from fresh material and at least one basidioma from each collection was longitudinally sectioned to observe its context. Basidiomata were dried in a hot air dehydrator (~45 °C) for their preservation. Spore prints were prepared in the field, placing the pilei spore-side down on aluminum foil sheets for 24 h. Colors were generically named and then coded according to Kornerup & Wanscher (1978) or Küppers (2002), with color plates noted in parentheses (e.g., 3A2 or $Y_{20}M_{10}C_{10}$). Details of the basidiomata surface were examined under a Zeiss Stemi 305 stereo microscope (Carl Zeiss AG, Oberkochen, Germany).

Micro-anatomical features were studied in a Zeiss Axioscope 5 microscope (Carl Zeiss AG) with bright field, phasecontrast optics, and a charge-coupled device camera (Zeiss Axiocam 208 color, Carl Zeiss AG) using sections of fresh or rehydrated tissues from the basidiomata. Dried samples were rehydrated in distilled water or moistened in ethanol 70%,

[3] Os números de registro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) referentes a manuscritos ainda não publicados serão devidamente cadastrados e informados apenas no momento do envio desses trabalhos para publicação, em conformidade com a legislação vigente.

and microsections were cut by hand with a razor blade. Microscopic structures were examined in water, 3–5% KOH, Congo red, Cotton Blue, Cresyl Blue, and Melzer's reagent, separately. At the beginning of a set of basidiospore data, the abbreviation [*a/b/c*] signifies "*a*" basidiospores measured from "*b*" basidiomata of "*c*" collections. Dimensions of basidiospores are presented in the following form *(m–)n–o(–p),* in which "*m*" is the smallest value observed or calculated and "*p*" is the largest value observed or calculated; in the range of values observed or calculated, the 5th percentile is "*n*" and the 95th percentile is "*o*". A summary of definitions of biometric variables follows:

L_m (W_m) = the average of all lengths (widths) of basidiospores measured.

Q = the ratio of length to width of a basidiospore or the range of such ratios for all basidiospores measured.

Q_m = the average of all Q values computed for all basidiospores measured.

The form of the basidiospores was interpreted based on the Q values following Bas (1969), and descriptive terms for other morphological features follow Vellinga (1988) and Vellinga & Noordeloos (2001). The width of the basidia was taken at the widest part and the length was measured from the apex without sterigmata to the basal septum. Measurements of basidiospores and other elements were obtained from digital photographs using the Zeiss Zen 3.5 Blue edition software (Carl Zeiss AG).

Newly Brazilian collections including the selected nomenclatural types are deposited at the fungarium IFungiLab (FIFUNGI) from the 'Instituto Federal de Educação, Ciência e Tecnologia de São Paulo' (IFSP) with some duplicates deposited at the herbarium SP from the 'Instituto de Pesquisas Ambientais' (IPA). Collections from Paraguay are deposited at the herbarium FACEN of the 'Universidad Nacional de Asunción' (UNA). The herbaria/fungaria abbreviations follow Thiers (2025, continuously updated).

DNA Extraction, PCR amplification, and sequencing

For the purpose of DNA extraction, the tissue fragment was excised from either fresh or dried basidiomata using a sterilized scalpel and tweezers. The procedure for the 'DNeasy Plant Mini Kit' (Qiagen, San Diego, California) and 'GenElute Plant Genomic DNA Miniprep Kit' (MilliporeSigma, Burlington, Massachusetts) DNA extraction kits were carried out according to the manufacturer's instructions.

The PCR reactions were conducted on an Mastercycler Nexus GX2 thermal cycler (Eppendorf SE, Hamburg, Germany). The following primers were used for amplification and sequencing: ITS1-F/ITS4 or ITS1/ITS4-B (White *et. al.* 1990, Gardes & Bruns 1993) for the

internal transcribed spacer (ITS: ITS1-5.8S-ITS2) fragment; LR0R/LR5 or LR0R/LR7 (Vilgalys & Hester 1990, James *et al*. 2006) for part of the nuclear ribossomal large subunit (nrLSU); Am-6F/Am-7R (Cai *et al*. 2014) for part of the second largest subunit of RNA polymerase II (*rpb2*); 983F/1567R (Rehner & Buckley 2005) for part of the Elongation Factor 1-alpha (*tef1-α*). The amplification reaction mixture contained 25 µl Taq Pol - Master mix (2×) Green (Cellco Biotech, São Paulo, Brazil), 2.5 µL each prime, 15 µL of ultrapure water, and 5 µL of template DNA. Thermal profile of PCR for rDNA markers was according to Binder & Hibbett (2003). To amplify the protein-coding genes, the touchdown PCR protocol following Justo & Hibbett (2011) was used. After visualization of positive PCR products on a 2% agarose gel with Safedye Nucleic Acid Stain (Cellco Biotech), PCR products were cleaned up prior to sequencing using QIAquick PCR Purification Kit (Qiagen) or PCR Purification Kit DPK-106 (Cellco Biotech) as per manufacturer's guidelines.

To get readable ITS sequences for the specimens NCC282 and NCC283, the DNA fragments were cloned using the pGEM-T Easy Vector System (Promega Co., Wisconsin, USA) in a 5:1 insert:vector ratio. Chemocompetent *Escherichia coli* Stellar cells were transformed with the ligation reactions. Bacterial cultures were plated in selective LB media containing ampicillin and incubated at 37 ºC for 16 h. Positive clones were confirmed by the digestion of the plasmid DNA with the endonuclease EcoRI (New England Biolabs, Massachusetts, USA). Two clones from each collection were sequenced using the universal primers pUC/M13F (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and pUC/M13R (5′-CAGGAAACAGCTATGAC-3′).

Sequencing (Sanger method) was carried out at the "Centro de Pesquisa sobre o Genoma Humano e Células-Tronco da Universidade de São Paulo" (CEGH-USP, São Paulo, Brazil) or at Macrogen Korea (Seoul, South Korea).

Alignment and phylogenetic analyses

Bidirectional sequencing results were assembled using Geneious Prime v2024.0.5 (Biomatters Ltd., Auckland, New Zealand). Low quality nucleotide sites at both ends of the sequences were manually pruned. Sampling for the phylogenetic backbone of *Macrolepiota* referred to Fazolino Perez *et al*. (2018), Freitas & Menolli (2019), Sysouphanthong *et al*. (2021), Souza *et al*. (2022), and Lebeuf *et al*. (2024). For this study, 49 ITS, 26 nrLSU, 20 *rpb2*, and 21 *tef1-α* sequences were generated. Additional sequences for the final datasets were downloaded from GenBank and included exemplars from 110 specimens from all three sections of *Macrolepiota*. Sequences of two species of *Leucoagaricus* [viz. *L. barssii* (Zeller) Vellinga,

L. meleagris (Gray) Singer] were used as outgroup taxa. All new sequences from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/). Table 1 gives all taxa, collection information, and GenBank numbers of sequences used in this study. The selected sequences were aligned using MAFFT v.7490 as implemented in Geneious (Katoh *et al*. 2002, Katoh & Standley 2013), adjusting the direction of nucleotide sequences according to the first sequence and selecting the E-INS-i iterative refinement method. Ambiguously aligned sites were manually eliminated, and the gaps were treated as missing data.

For assessment of the infrageneric relationships of the studied species, two datasets were individually analyzed. One dataset comprising sequences of ITS, nrLSU, *rpb2*, and *tef1-α* (dataset I), and the other containing only ITS sequences (dataset II). To build the multilocus phylogenetic tree (Figure 2), individual gene alignments were concatenated using Mesquite v.3.70 (Maddison & Maddison 2023). The latter software was also used to convert the type and extension of the files between the different steps of the workflow. The Dataset I was subdivided into seven data partitions: ITS-spacers, 5.8S, nrLSU, *rpb2*-exons, *rpb2*-intron, *tef1-α*-exons, and *tef1-α*-introns. Conflicts between the four genes were tested using PAUP* v.4.0b10 (Swofford 2002). The results of the phylogenetic signals in the Dataset I were not in conflict.

The phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian inference (BI) via Cipres Scientific Gateway (https://www.phylo.org/portal2/home.action) (Miller *et al*. 2010). Maximum likelihood bootstrap analyses for phylogeny and assessment of branch support by bootstrap (BS) percentages were carried out using the RAxML-HPC v.8 on XSEDE (8.2.12) (Stamatakis 2014) under the GTRCAT model with 25 per site rate categories, along with 1000 rapid bootstrap repetitions (Felsenstein 1985). Bayesian inference analyses (BI) for the reporting of Bayesian posterior probabilities (BPP) support for branches were performed using MrBayes on XSEDE (3.2.7a) (Huelsenbeck & Ronquist 2001, Ronquist *et al*. 2012). The best-fit nucleotide substitution model for each gene partition was determined with jModelTest2 v.2.1.6 on XSEDE (v. 3.2.6) (Posada & Crandall 1998) via the Cipres Science Gateway (Miller *et al*. 2010) based on the Bayesian Information Criterion (BIC). Four simultaneous Markov chains were run for 10 million generations and trees were sampled every 1,000th generation (resulting in 10,000 trees). The first 25% trees, which represent the burn-in phase of the analyses, were discarded, while the remaining ones were used for calculating the consensus tree and BPPs. To test the convergence and stability of the runs, the average standard deviation of split of frequencies (< 0.01) was evaluated in Tracer 1.6 (Rambaut *et al*. 2018).

Phylogenetic trees from ML analyses were visualized in FigTree v1.4.4 (Rambaut 2018)] and edited in CorelDRAW 2024 (Alludo, Ottawa, Canada) and Adobe PhotoShop CC 2024 (Adobe Systems, São José, California) for figure presentation and to complement with the

support values of the BI analyses. Branches that received BS and BPP greater than or equal to 70% and 0.95, respectively, were considered to be significantly supported.

Area of the ethnomycological study

The semi-rural district of Parelheiros is situated in the southernmost region of the city of São Paulo, the largest urban center in Brazil (Figure 1). With an approximate area of 153 km², it is one of the largest districts in the city, though with the second-lowest population density (855 inhabitants/km^2) (IBGE 2023). The district territory consists largely of protected springs, wetlands, and Atlantic Rainforest remnants, maintaining a large part of its native forest (62,4 %), as well as preserved biodiversity and an area of large agricultural production. Parelheiros has a humid subtropical climate type that is characterized by hot and humid summers and cool to mild winters. This climate is strategic for the whole city environment, as it balances the thermal currents with its milder average temperature (17.3 ºC) and its high annual rainfall (2027 mm) with precipitation accumulation from October to March (Sepe & Takiya 2004, Prefeitura Municipal de São Paulo 2019). Its water supply network includes three river basins: Capivari, Guarapiranga and Billings, which collectively provide water to approximately 25% of the population in the metropolitan region of São Paulo. (Prefeitura Municipal de São Paulo 2019).

Table 1. Taxa, vouchers, locations, and GenBank accession numbers of the specimens used in the molecular phylogenetic analyses. Sequences in bold were newly generated. Sample numbers followed by (T) refer to type specimens.

Taxon	Sample no.	Country	GenBank accession No.			
			ITS	nrLSU	*rpb2*	*tef1-α*
Macrolepiota aberdarense	MAT08 (**T**)	Kenya	KP974615	KP974624	-	-
M. aberdarense	RAG04	Kenya	KP974616	KP974625	-	-
M. abruptibulbosa	NCC278 (T)	Brazil	**This study**	**This study**	**This study**	**This study**
M. abruptibulbosa	NCC186	Brazil	**This study**	**This study**	**This study**	**This study**
M. abruptibulbosa	NCC295	Brazil	**This study**	**This study**	-	-
M. abruptibulbosa	iNat #215959597	Brazil	**This study**	-	-	-
M. abruptibulbosa	iNat #222645313	Brazil	**This study**	-	-	-
M. abruptibulbosa	iNat #211097848	Brazil	**This study**	-	-	-
M. abruptibulbosa	iNat #211096291	Brazil	**This study**	-	-	-
*M. bonaerensis****	Faz362	Brazil	KY927718	KY907188	-	-
*M. bonaerensis****	Faz148	Brazil	KY927717	KY907187	-	-
*M. bonaerensis****	Faz643	Brazil	KY927720	KY907190	-	-
M. bonaerensis	MPD477	Brazil	**This study**	**This study**	**This study**	**This study**
M. bonaerensis	NCC183	Brazil	**This study**	**This study**	**This study**	**This study**
M. bonaerensis	NCC269	Brazil	**This study**	**This study**	-	-
M. bonaerensis	NCC270	Brazil	**This study**	**This study**	-	-

M. bonaerensis	NCC288	Brazil	**This study**	**This study**	-	-
M. bonaerensis	NCC290	Brazil	**This study**	**This study**	-	-
M. bonaerensis	NCC289	Brazil	**This study**	**This study**	-	-
M. bonaerensis	MC294	Paraguay	**This study**	-	-	-
M. bonaerensis	MC800	Paraguay	-	**This study**	-	-
M. bonaerensis	Neves M.A. 752	Brazil	**This study**	-	-	-
M. bonaerensis	DER 574	Brazil	**This study**	-	-	-
M. bonaerensis	MAC 330	Brazil	**This study**	-	-	-
M. bonaerensis	CHC54	Brazil	**This study**	-	-	-
M. bonaerensis	CHC55	Brazil	**This study**	-	-	-
M. capelariae	DAZ060 (T)	Brazil	ON753531	ON794497	**This study**	**This study**
M. capelariae	MC4584	Brazil	ON753533	OP302841	**This study**	**This study**
M. capelariae	MC4588	Brazil	ON753532	OP302842	**This study**	**This study**
M. capelariae	DSF02	Brazil	ON753529	-	-	-
M. capelariae	ADF04	Brazil	ON753530	-	-	-
M. capelariae	AL228	Brazil	ON753534	-	-	-
M. capelariae	DAZ195	Brazil	**This study**	-	-	-
M. capelariae	NCC184	Brazil	**This study**	-	-	-
M. capelariae	NCC185	Brazil	**This study**	-	-	-
M. capelariae	FB187	Brazil	**This study**	-	-	-
M. capelariae	MPD726	Brazil	**This study**	-	-	-
M. capelariae	MIND.Funga0788	Brazil	**This study**	-	-	-
M. capelariae	CHC12	Brazil	**This study**	-	-	-
M. capelariae	NCC291	Brazil	**This study**	-	-	-
*M. capelariae****	GA73	Argentina	MN847716	-	-	-
*M. capelariae****	MO/144260	Mexico	MH290361	-	-	-
M. capelariae var. *velana*	DAZ105	Brazil	**This study**	**This study**	**This study**	**This study**
M. capelariae var. *velana*	NCC202	Brazil	**This study**	**This study**	**This study**	**This study**
M. chapeleta	NCC155 (T)	Brazil	**This study**	**This study**	**This study**	**This study**
M. chapeleta	NCC203	Brazil	**This study**	**This study**	**This study**	**This study**
M. chapeleta	DAZ200	Brazil	**This study**	-	-	-
M. chapeleta	EC051	Paraguay	**This study**	-	-	-
M. chapeleta	YM183	Paraguay	**This study**	**This study**	-	-
M. chapeleta	NCC187	Brazil	**This study**	-	-	-
M. chapeleta	NCC294	Brazil	**This study**	-	-	-
M. chapeleta	BDIF908	Brazil	**This study**	-	-	-
*M. chapeleta****	SB80	Argentina	MN847715	-	-	-
*M. chapeleta****	SB194	Argentina	MN847714	-	-	-
*M. chapeleta****	Faz563	Brazil	KY927719	KY907189	KY964600	-
*M. chapeleta****	Rother124	Brazil	KY927724	-	-	-
*M. chapeleta****	Rother126	Brazil	KY927725	-	-	-
*M. chapeleta****	ACM1167	Brazil	KY927726	KY907194	-	-
M. clelandii	Kok 788	Australia	AY083195	-	-	-
M. clelandii	Thiele 2650	Australia	AF482838	AF482882	-	-
M. clelandii	MEL2046436	Australia	AY083198	-	-	-
M. clelandii	not informed	New Zealand	AY083196	-	-	-
M. colombiana	NY EFM694 (T)	Colombia	U85311	U85276	-	-
M. cyanolamellata	ICN 187662 (T)	Brazil	NR_163288	NG_066387	-	-

M. cyanolamellata	ICN 187662	Brazil	KY927713	KY907183	-	-
M. cyanolamellata	ICN 187663	Brazil	KY927714	KY907184	-	-
M. cyanolamellata	NCC292	Brazil	**This study**	**This study**	**This study**	**This study**
M. cyanolamellata	MC518	Paraguay	**This study**	**This study**	-	**This study**
M. detersa	HKAS 55306 (T)	China	NR_119832	-	-	-
M. detersa	NIBRFG0000131193	South Korea	MK453226	-	-	-
*M. detersa****	8-X-1999	Japan	AY243586	-	-	-
M. detersa	MFLU121784	Thailand	KJ524560	-	-	-
M. dolichaula	HAI-37	Thailand	JQ683120	-	-	KC884712
M. dolichaula	MFLU121820	Thailand	KJ524564	-	-	-
M. dolichaula	HNL503277	Laos	MW542997	-	-	-
M. dolichaula	Canning 6603	Australia	AF482839	-	-	-
M. dunensis	NMJ161	Brazil	MG136892	-	-	-
M. eucharis	Bayliss E4505	Australia	AF482854	-	-	-
M. excoriata	Vellinga 20-X-1997	France	AY243607	-	-	-
M. excoriata	HAI-347	Israel	JQ683100	-	-	KC884713
M. excoriata	HAI-C-992	Israel	JQ683089	-	-	KC884720
M. excelsa	HNL501921	Laos	MW541837	-	-	-
M. excelsa	ecv3553	Thailand	KC920645	-	-	-
M. excelsa	ecv3572 (T)	Thailand	KC920644	-	HM488861	-
M. fuliginosa	20-IX-1990	Germany	AY243597	-	-	-
M. fuliginosa	P.H. Kelderman	Netherlands	AY243598	-	-	-
*M. fuliginosa****	P.D. Orton 3666	UK	AY243596	-	-	-
M. gracilenta	LE 9866	Russia	JQ683122	-	-	-
M. konradii	Vellinga 2292	Netherlands	AY243603	-	-	-
M. konradii	E.C. Vellinga 2289	Netherlands	AY243602	-	-	-
M. konradii	M. Groenendaal (L)	Netherlands	AY243601	-	-	-
M. macilenta	iNat #33915776	USA	MZ667912	-	-	-
M. macilenta	iNat #17734320	USA	MZ667911	-	-	-
M. macilenta	iNat #15930352	USA	MZ667908	-	-	-
M. macilenta	iNat #34175553	USA	MZ667901	-	-	-
M. macilenta	iNat #33910816	USA	MZ667899	-	-	-
M. macilenta	iNat #130021186	USA	OP643321	-	-	-
M. macilenta	iNat #86938953 (T)	USA	OK490279	-	-	-
M. macilenta	iNat #17936460	USA	MZ667898	-	-	-
M. mastoidea	18-IX-1990 Vellinga	Germany	AF482844	-	-	-
M. mastoidea	HKAS51950	China	HM125532	-	-	-
*M. mastoidea****	CBS169 46	France	U85314	U85279	-	-
*M. mastoidea****	CBS452.79	France	U85313	U85278	-	-
M. mastoidea	HKAS 50194	China	HM125531	-	-	-
M. mastoidea	HKAS 11207	China	HM125529	JN940272	JN993698	-
M. mastoidea	HKAS 11084	China	HM125530	JN940271	JN993701	-
M. mastoidea	7-XI-1998 N.J. Dam	Netherlands	AY243604	-	-	-
*M. mastoidea****	16-XI-1991 JG	France	AY243600	-	-	-
*M. mastoidea****	26-XI-1995 JG	France	AY243599	-	-	-
M. orientiexcoriata	HKAS 45863 (T)	China	NR_119833	JN940279	JN993682	-
M. orientiexcoriata	HKAS 49001	China	HM125524	JN940278	JN993695	-
M. orientiexcoriata	HMAS73304	China	HM125527	-	-	-
M. orientiexcoriata	HMAS63157	China	HM125526	-	-	-
M. orientiexcoriata	HKAS23040	China	HM125525	-	-	-

M. pallida	iNat #15945117	USA	MZ667907	-	-	-
M. pallida	S.D. Russell HRL1765	Canada	MH979449	-	-	-
M. pallida	S.D. Russell HRL1147	Canada	MH979426	-	-	-
M. pallida	DAOM 984979 (T)	Canada	ON287029	-	-	-
M. pernuda	CT03	Brazil	**This study**	**This study**	**This study**	**This study**
M. pernuda	NCC205	Brazil	**This study**	**This study**	**This study**	**This study**
M. pernuda	NCC201	Brazil	**This study**	**This study**	**This study**	**This study**
M. pernuda	NMJ279 (T)	Brazil	**This study**	**This study**	**This study**	**This study**
M. phaeodisca	P. Roux 994	France	AF482847	-	-	-
M. procera	HKAS 5722	China	HM125513	JN940275	JN993696	-
M. procera	HKAS 8108	China	HM125514	JN940277	JN993697	-
M. procera	Boekhout 1049	Netherlands	AY243588	-	-	-
M. procera	HAI 23	Israel	JQ683108	-	-	KC884724
M. procera	HAI 72	Israel	JQ683106	-	-	KC884725
M. procera	LOU-Fungi 18703	Italy	HQ423288	-	-	HM488892
M. procera	LOU-Fungi 18695	Italy	HQ423287	-	-	-
M. psammophila	LE 241971	Israel	JQ683115	-	-	-
M. psammophila	HAI C 1187	Israel	JQ683096	-	-	KC884705
M. psammophila	HAI SP 14	Israel	JQ683085	-	-	KC884711
M. pulchella var. *pulchella* ***	Faz683	Brazil	KY927721	KY907191	KY964601	-
M. pulchella var. *pulchella* ***	Faz684	Brazil	KY927722	KY907192	KY964602	-
M. pulchella var. *pulchella* ***	Faz685	Brazil	KY927723	KY907193	KY964603	-
M. pulchella var. *pulchella*	JJSO386	Brazil	MG136891	-	-	-
M. pulchella var. *pulchella*	MC4411	Brazil	MG136894	-	-	-
M. pulchella var. *gymnopodia*	NCC287	Brazil	**This study**	-	-	-
M. pulchella var. *gymnopodia*	NCC178 (T)	Brazil	**This study**	**This study**	**This study**	**This study**
M. pulchella var. *gymnopodia*	NMJ284	Brazil	**This study**	**This study**	**This study**	**This study**
M. sabulosa	UFRN-Fungos 2693 (T)	Brazil	KY927715	KY907185	KY964598	-
M. sabulosa	UFRN-Fungos 2694	Brazil	KY927716	KY907186	KY964599	-
M. sabulosa	NCC282	Brazil	**This study**	**This study**	**This study**	**This study**
M. sabulosa	NCC283	Brazil	**This study**	**This study**	**This study**	**This study**
M. sabulosa var. *velistellaris*	NMJ185 (T)	Brazil	MG136893	-	-	-
M. subcitrophylla	HKAS 58248	China	JN180322	-	-	-
M. subcitrophylla	HKAS 61624 (T)	China	JN180321	-	-	-
M. turbinata	H0219 (T)	Australia	NR_119949	NG_042584	-	-
M. turbinata	DunstanL055	Australia	JF495072	JF495033	-	-
M. umbonata	SFC20160909-16 (T)	South Korea	MK453245	-	-	-
M. umbonata	SFC20150819-27	South Korea	MK453242	-	-	-
M. velosa	HKAS 29487 (T)	China	NR_119459	-	-	-
M. velosa	HKAS2172	China	AF482853	-	-	-
M. vinaceofibrillosa	H0047	Australia	JF495070	-	-	-
Macrolepiota sp.	MO/306986	Mexico	PP724363	-	-	-
Macrolepiota sp.	JLF10816	USA	OP522352	-	-	-
Macrolepiota sp.	iNat #131272162	USA	OR583681	-	-	-

Macrolepiota sp.	iNat #94815636	USA	PP274088	-	-	-
Macrolepiota sp.	iNat #94815088	USA	PP274087	-	-	-
Macrolepiota sp.	MO/245133	Mexico	MH231156	-	-	-
Outgroup						
Leucoagaricus barssii	AFTOL ID 1899	USA	DQ911600	DQ911601	DQ911602	-
L. meleagris	30-VII-1996 Vellinga	Netherlands	AY176419	AF482890	-	-

Note: *** indicates taxon names modified according to Vizzini *et al*. (2011), Souza *et al*. (2022) and this study.

The establishment of Parelheiros district began in 1829 with the arrival of 94 German immigrant families in the region, which was already inhabited by Tupi indigenous and traditional caboclo communities (Prefeitura Municipal de São Paulo 2019). By mid-1940, the region began to receive Japanese immigrants that came to explore agriculture and helped in the region development, transforming Parelheiros into one of the largest agricultural areas in São Paulo city (Prefeitura Municipal de São Paulo 2019). Nowadays, descendants of German and Japanese immigrants are still a significant contingent in the demographic structure of the Parelheiros district (Prefeitura Municipal de São Paulo 2019).

Ethnomycological data collection and analysis

The fieldwork was carried out in the rainy season from December to April 2023/2024 in Parelheiros district, an area pertains to the Atlantic Rainforest bioregion. A total of 15 informants (9 male and 6 female) from Parelheiros were involved in this study. Additionally, seven informants from different locations in Southeastern and Southern Brazil (1. Camanducaia, in the state of Minas Gerais; 2. São Francisco de Paula, in the state of Rio Grande do Sul, and 3. Itaiópolis, in the state of Santa Catarina) were involved to support and corroborate the information about common edible *Macrolepiota* species. The final 22 informants were classified into two categories: key informants (6) and general informants (16). Criteria for nominations of a participant as key respondents included: (1) have a profile on a social network that shares information on local collection and consumption of wild edible mushrooms (WEM), and (2) informants who, prior to this investigation, had already sent collections of edible *Macrolepiota* to the lead author's workplace, requesting identification. The remaining informants were selected based on the snowball method described by Bernard (2006) and Albuquerque *et al*. (2014).

The ethnomycological data were collected from a very close interaction with informants using semi-structured interviews, focus group discussions, participant observation, and walk-in-the-woods methods (Vasco-Palacios et al. 2008, Martin 1995). Semi-structured interviews

were carried out with all informants. Individual contact to schedule the interviews and participant observation was made according to the consent of each informant and their time availability. During the interviews, socio-demographic profile and themes related to the knowledge of wild *Macrolepiota* mushrooms, including naming, habitat, collection, seasonality, uses (fresh/dried), methods of preparation for food, preservation (storage), and marketability were recorded. Dried and/or fresh specimens and colored photographs of representative *Macrolepiota* specimens were used during interviews and discussions with all respondents. The interviews were audio-recorded and fully transcribed for speech recording and subsequent analysis.

Independent walk-in-the-woods and participant observations were employed with all key informants for practical identification of wild *Macrolepiota* taxa in the field and to corroborate the information on collection practices, species recognition, and use of vernacular names. In the focus group discussions, key and general informants were invited to share their experiences on consuming wild *Macrolepiota* specimens, as well as to cooperate in the preparation of receipts using different species of *Macrolepiota*. This last proceeding was carried out to support the information gathered from previous individual approaches.

All collected data underwent analysis through a process of reviewing the content of the interviews, participant observations, and focus groups. The content analysis aimed at identifying empirical categories was carried out following these steps: pre-analysis, material exploration, and processing and interpretation of the obtained results (Bardin 2016). Descriptive statistics using frequencies and percentages were used to summarize data using Microsoft Excel 2024 (Microsoft Corporation, Redmond, Washington).

All *Macrolepiota* samples referred as edible and gathered in the focus group discussions, walk-in-the-woods forays, participant observations, and from the independent informant collections were processed in the field following standard methods (Souza *et al*. 2020) and preserved for further studies. The identification based on morphotaxonomy, ecology, and multigene phylogenetic analyses was performed at the IFungiLab – 'Laboratório de Ensino, Pesquisa e Extensão em Micologia' in the 'Instituto Federal de Educação, Ciência e Tecnologia de São Paulo' – IFSP (São Paulo, Brazil). All identified specimens are deposited at the IFungiLab fungarium (FIFUNGI) from the same institution (Thiers 2024, continuously updated).

The data presented in this ethnomycological report are part of the results of the projects: "Diversity and use of wild edible mushrooms by local communities and restaurants in southern and southeastern Brazil" and "Ethnomycological knowledge of Santa Catarina's northern plateau", which both have authorization from the Research Ethics Committee of the 'Instituto

Federal de Educação, Ciência e Tecnologia de São Paulo' – CEP-IFSP (CAAE: 74709623.7.0000.5473) and of the 'Universidade Federal de Santa Catarina' – CEPSH-UFSC (CAAE: 74364723.4.0000.0121). All informants signed a Free Informed Consent Form (FICF), in compliance with Resolution 466/2012 of the 'Comissão Nacional de Ética e Pesquisa do Ministério da Saúde'.

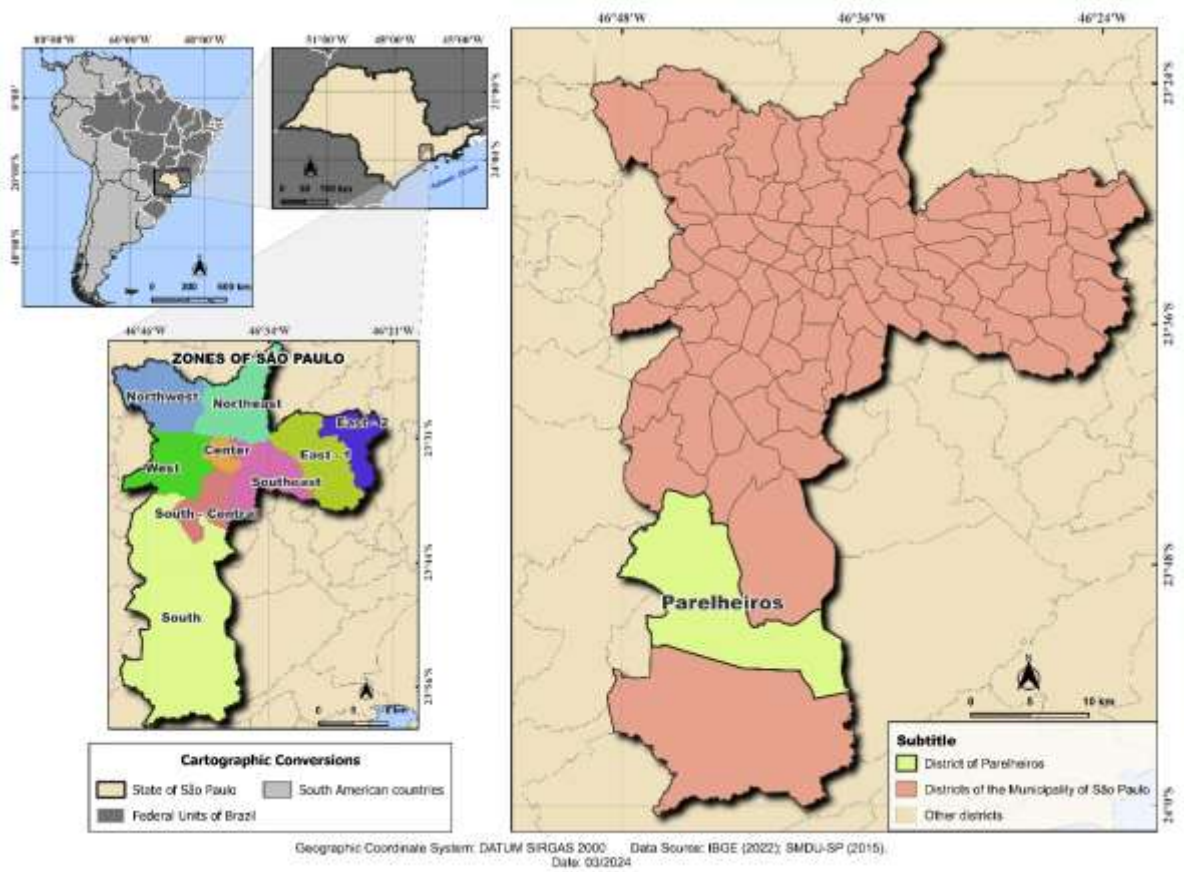

Figure 1. Map showing the main area of the ethnomycological study: district of Parelheiros, south region of the city of São Paulo, São Paulo state, Brazil.

Proximate nutritional analysis

For sample preparation, 50 g of dehydrated mushrooms of *M. bonaerensis*, *M. capelariae*, and *M. chapeleta* were gathered during ethnomycological inventories in the Parelheiros district. These species were the most frequently and abundantly represented in the district, yielding up to 1 kg of fresh basidiomata per collection point. The remaining edible *Macrolepiota* species considered in the ethnomycological part were not evaluated at the nutritional level due to an insufficient number of basidiomata sampled, which precluded a meaningful assessment.

The proximate characterization of the samples was carried out at the Bromatology Laboratory of the Animal Production Department at 'Universidade Estadual Paulista Julio de

Mesquita Filho' – UNESP, in Dracena (Sao Paulo state, Brazil) according to the methodologies of the Adolfo Lutz Institute (https://www.ial.sp.gov.br/). The moisture content was determined by dehydrating the samples in an oven with forced air circulation at 105 °C for 12 h or until constant weight (method 012/IV) (Zenebon *et al*. 2008). Crude fibre was determined by acid and alkaline digestion and subsequent heating of 2 g sample in a Thermolyne® Small Benchtop Muffle (Thermo Fisher Scientific Inc., Waltham, USA) at 550 ºC to produce ash of gray color. The desiccator was used for cooling, and the sample was weighed (method 044/IV) (Zenebon *et al*. 2008). The ash content was determined by subjecting a 3 g sample to incineration in a muffle furnace (branded above) at 550 °C for six h (method 018/IV) (Zenebon *et al*. 2008). Crude protein was evaluated by the macro-Kjeldahl apparatus (method 036/IV, Zenebon *et al*. 2008) using the conversion factor of 4.38 [Crude protein (%) = 4.38 × Nitrogen (%)], which is used for fungi (Crisan & Sands 1978, Krishnamoorthi *et al*. 2022). Soxhlet extraction with petroleum ether was used for the gravimetric evaluation of fat content (method 321/IV, modified). The total of carbohydrates was calculated by the difference method as follows: carbohydrates = 100 - (moisture + ash + crude fat + crude protein + crude fiber) (Colak *et al*. 2007). Determination of total energy value was according to the given equation: energy (kcal) = 4 × (g crude protein + g carbohydrate) + 9 × (g crude fat) (Grimm *et al*. 2021, Lee & Cho 2021). All experiments were carried out in triplicate. The results were expressed in dry weight percentage and presented as the mean (μ) ± standard deviation (σ) of the three parallel measurements.

Determination of mineral elements

The estimation of the mineral content of samples previously subjected to proximate analysis was conducted at the Laboratory of Plant Nutrition at the Faculty of Engineering at 'Universidade Estadual Paulista Júlio de Mesquita Filho' – UNESP, in Ilha Solteira city (São Paulo state, Brazil). The content of macrominerals (P, K, Ca, and Mg) and microelements (Cu, Fe, Mn, and Zn) was determined using spectrophotometric methods according to Malavolta *et al*. (1997) and Silva (2009).

RESULTS

Phylogenetic analyses

The combined multilocus matrix (dataset I), consisted of 229 sequences. The final alignment contained 2,928 nucleotide site including gaps (738 sites for ITS, 843 sites for nrLSU, 757 sites for *rpb2*, and 586 sites for *tef1-α*), of which 2172 were conserved, 114 were parsimony-informative, and 642 were variable but parsimony-uninformative. For BI, the best-fit evolutionary model for each region of four markers were TPM2uf+G (ITS-spacers), JC (5.8S), TrN+I (nrLSU), K80+I (*rpb2*-exons), HKY (*rpb2*-intron), TrNef+G (*tef1-α*-exons), and K80+G (*tef1-α*-introns). No obvious differences in the topology and statistical support were observed between the ML and BI analyses, except by the phylogenetic placement of the clade containing *M. subcitrophylla* Z.W. Ge, in Ge, Chen & Yang and *M. clelandii* Grgur., which changed to be basal within *Macrolepiota* sect. *Macrolepiota* in the BI tree. The ML topology is presented as a master tree (Figure 2).

In the boundaries delineated here, *Macrolepiota* was composed of three mains significantly supported clades, corresponding to *Macrolepiota* sect. *Macrolepiota* (MLB = 90 %), *Macrolepiota* sect. *Macrosporae* (MLB = 100 %, BPP = 1.00), and *Macrolepiota* sect. *Volvatae* (MLB = 100 %, BPP = 0.98), which is in agreement with previous phylogenetic analyses of *Macrolepiota* (viz. Fazolino Perez *et al*. 2018, Sysouphanthong *et al*. 2021, and Lebeuf *et al*. (2024), although *Macrolepiota* sect. *Macrolepiota* has received low to subsignificant support in several previous reconstructions (Ge *et al*. 2010, 2012, Fiaz *et al*. 2014, Souza *et al*. 2022). Among the recovered phylospecies, four lack Latin binomials that can be unambiguously assigned to them and thus represent new putative species. Three of these novelties are formally introduced below (see Taxonomy) as *M. abruptibulbosa*, *M. chapeleta*, and *M. pernuda*, while the remaining one requires further morphological characterization before it can be named or formally described, and it is here provisionally referred to as *Macrolepiota* sp. (MO/245133).

The clades with sequences of *M. pernuda* from Brazil and *M. bonaerensis* from Brazil and Paraguay were both equally well-supported (MLB = 98 %, BPP = 0.99), with *M. pernuda* closely nest to *M. colombiana* from Colombia and the *Macrolepiota* sp. (MO/245133) from Mexico. All these species formed a strong supported lineage (BS = 93 %, BPP = 1.00) that is named here as /colombiana clade. Sampled collections in this clade present both pairwise distances of ITS sequences lower than 3% and several overlapping suites of morphological traits. Thus, they are therefore treated as a species complex, viz. *M. colombiana* complex.

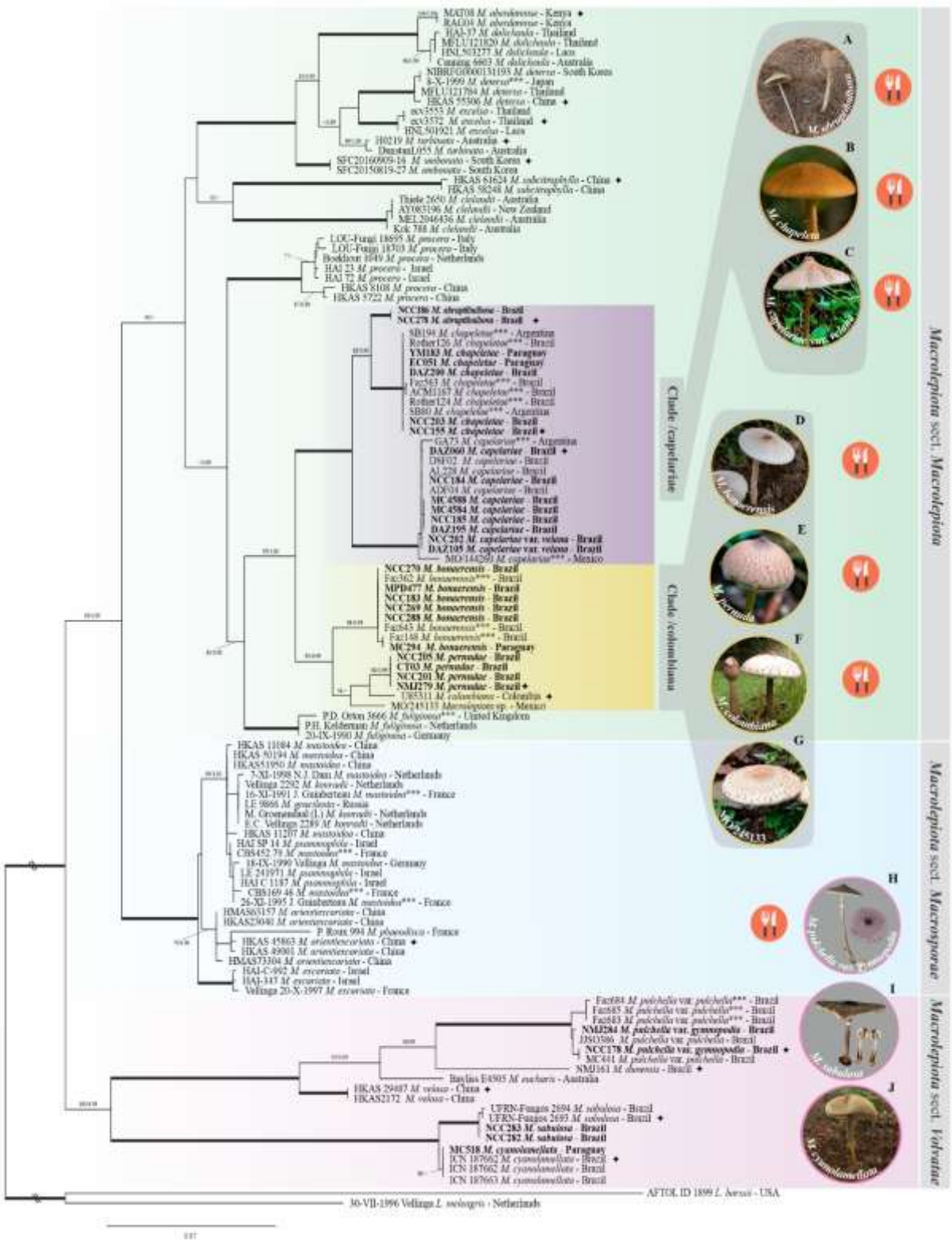

Figure 2. Combined phylogenetic ITS-nrLSU-*rpb2*-*tef1*-*α* (dataset I) topology from ML analysis for the genus *Macrolepiota*. Nodes were annotated if supported by ≥ 70 % ML BP (left) or ≥ 0.95 Bayesian PP (right). Thick lines on branches indicate 100 BP and 1.00 BPP supports. The three recovered sections and the clades /capelariae and /colombiana are presented under color highlight. The tree is rooted to *Leucoagaricus barssii* (AFTOL ID 1899) and *L. meleagris* (30-VII-1996 Vellinga). Collections sequenced in the present study are highlighted

in boldface. Black stars in front of the terminal names represent holotype specimens. Photos of some commented species of *Macrolepiota* from the Neotropics are depicted in right side. *** indicates taxon names modified according to Vizzini *et al.* (2011), Souza *et al.* (2022), and the present study. Edible species unveiled in this study are indicated with a symbol (fork and knife) beside the respective photo. Photographs: **A**. J.M. Timm, **B**−**D**, **H**. C. Coelho-Nascimento, **E**. N. Menolli Jr., **F**. A.E. Franco-Molano, **G**. ©Alan Rockefeller (iNaturalist), **I**. P.G.R Xavier, **J**. M.G. Campi.

The Brazilian and Paraguayan sequences of *M. chapeleta*, as well as sequences of two matching collections from Argentina, formed a fully supported clade (MLB = 100 %, BPP = 1.00) that resides in sister position to *M. abruptibulbosa*, a species so far known from two collections in high elevation *Pinus* spp. forest of Southern Brazil. The clade '*M. chapeleta* + *M. abruptibulbosa*', in turn, is sister to the *M. capelariae* clade that includes samples from Brazil, Argentina, and Mexico. All three clades form the /*capelariae* lineage with full support (MLB = 100 %, BPP = 1.00). The pairwise divergence of the ITS marker in the sequences within this lineage ranged from 0.16 to 4.75%. Furthermore, the clades /*capelariae* and /*colombiana* are sister to each other, forming a superclade that mostly includes species from the Neotropics, which is sister to *M. fuliginosa* (Barla) Bon, originally described from France.

The clade of *M. cyanolamellata* and *M. sabulosa* that was recovered within *Macrolepiota* sect. *Macrolepiota* in previous studies (Fazolino Perez *et al*. 2018, Freitas & Menolli 2019, Sysouphanthong *et al*. 2021, Souza *et al.* 2022) appeared with a distinct placement in our analyses (Figure 2). It was clustered with maximum support within the *Macrolepiota* sect. *Volvatae* core clade that is composed of the volvate taxa *M. dunensis*, *M. eucharis* Vellinga & Halling*, M. pulchella*, and *M. velosa* Vellinga & Zhu L. Yang. The latter placement seems to be more adequate because species in *Macrolepiota* sect. *Volvatae* are characterized by having a volva at the stipe base. It is noteworthy that newly collections of *M. sabulosa* (NCC282, NCC283) from the type locality exhibited a limbate membranous volva at the stipe base, which was not previously mentioned in the protologue but was observed in the re-examination of the type specimen (see discussion under Taxonomy). Similarly, in *M. cyanolamellata*, although the presence of a volva is neither shown in the type illustrations nor mentioned in the protologue, the analysis of recent collections (MC518 and NCC292) and the re-examination of the type specimens (ICN 187662 and ICN 187663) confirmed the presence of a distinct basal volva (see discussion under Taxonomy).

The Dataset II was based on the backbone of the ITS sequences from Dataset I, including almost all newly generated sequences plus 17 sequences of *Macrolepiota* from North America

(Canada, México, and the USA) primarily generated by ITS barcoding initiatives of collections available on iNaturalist and recently addressed in Lebeuf *et al*. (2024). The aligned dataset contained 114 terminals, and the same outgroup from Dataset I was used. It consisted of 750 characters including gaps, of which 475 were conserved, 44 were parsimony-informative, and 231 were variable but parsimony-uninformative. For BI, the substitution models were as follows: HKY+I+G (ITS1), JC (5.8S), and SYM+G (ITS2). The phylogenetic trees generated by BI an ML analyses were largely congruent. The ML topology is presented as master tree (Figure 3). The overall topology of *Macrolepiota* obtained from Dataset II is consistent with that of previous works conducted by Fazolino Perez *et al*. (2018), Freitas and Menolli (2019), Sysouphanthong *et al*. (2021), and Souza *et al.* (2022).

The sequences from North American materials were recovered in four well-supported clades (namely clades 1 to 4 in Figure 3). Clade 1 comprises a single collection from Mexico MO 306986 (= iNat #194406677) that is sister to *M. capelariae* and most likely represents an undescribed species. It bears a strong resemblance to *M. capelariae*, particularly in the pileus covering, but produces a complex ascending annulus that is fibrillose upperside and with a squamulose edge (Tux 2023), which is unique among species in the /capelariae clade. In addition, it has a densely appressed-squamulose stipe covering that is only comparable to that observed in *M. sabulosa* and *M. cyanolamellata*. It is pertinent to note that other specimens phenotypically corresponding to the collection MO 306986 (= iNat #194406677) are commonly observed in Mexico from records of the iNaturalist, corroborating the distinctive suite of traits in question is consistent across other populations (Figure 4). Clade 2 (MLB = 99 %, BPP = 1.00) encompasses four collections from the state of Arizona (USA) and one from Oaxaca, southern Mexico. The collections of this clade, which macromorphologically resemble the well-known *M. bonaerensis* from South America (Rockefeller 2016, Torres-Grant 2021a, 2021b, Frank 2022, Singer 2022), are sister to the clade containing *M. bonaerensis*, *M. colombiana*, and *M. pernuda* within the /colombiana clade. Clade 3 (MLB = 99%, BPP = 1.00) harbors eight collections from the USA and represents a recently described species, *M. macilenta*. This clade clusters as sister to clade 4, which also represents a recently described species from Canada and the USA, namely *M. pallida*. The ‘clade 3 + clade 4’ received a strong support (MLB = 93 %, BPP = 0.98) and is sister to the large lineage that includes either /capelariae and /colombiana clades.

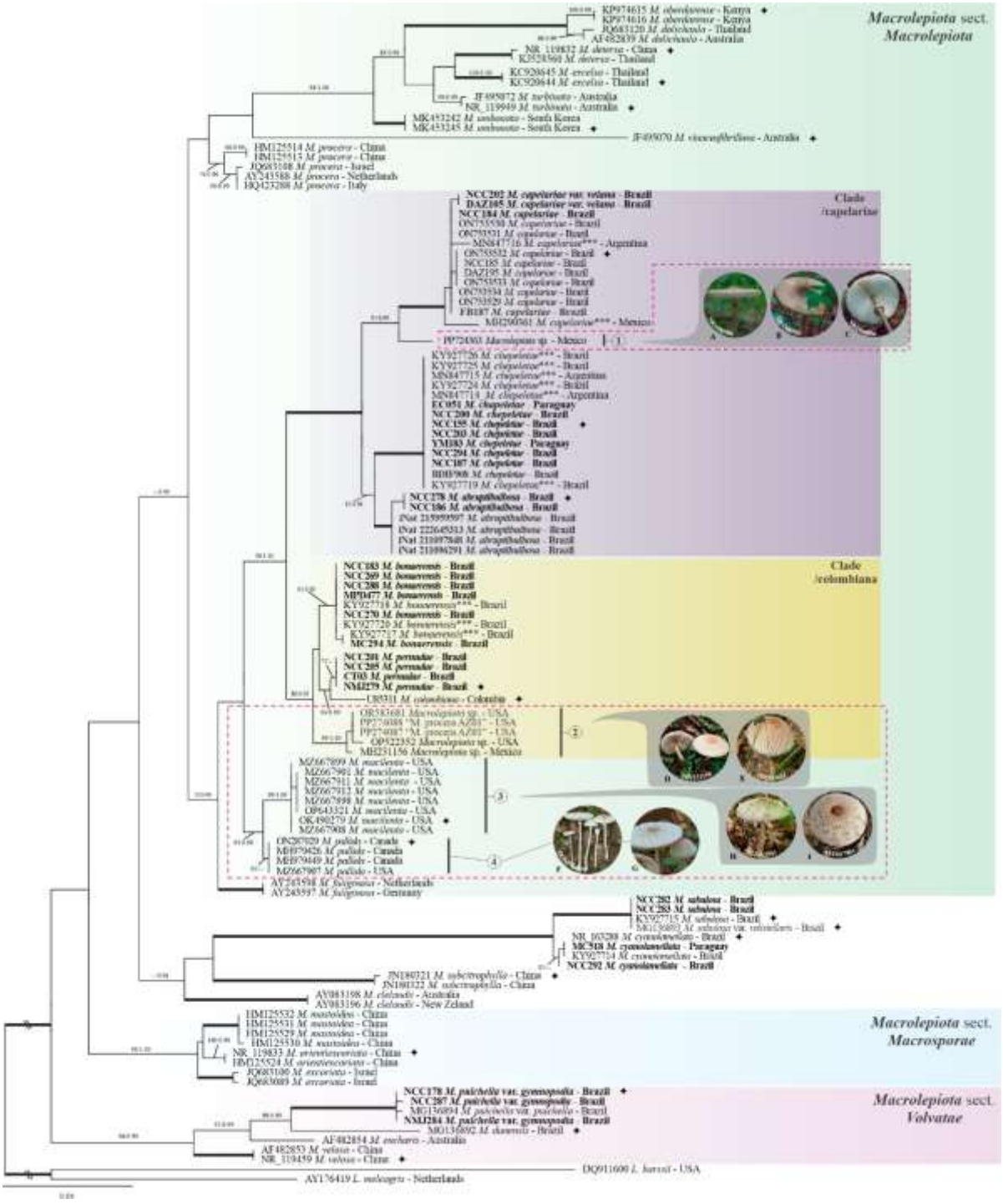

Figure 3. ITS-based phylogram from ML analysis of the genus *Macrolepiota* (dataset II). Nodes were annotated if supported by ≥ 70% MLB (left) or ≥ 0.95 BPP (right). Thick lines on branches indicate 100 MLB and 1.00 BPP supports. The three recovered sections and the clades /capelariae and /colombiana (= *M. colombiana* complex) are presented under color highlight. Clades harboring North American sequences of collections from iNaturalist, Mushroom Observer, and Lebeuf *et al*. (2024) are numbered from 1–4 and highlighted in a red dashed square along with the photos representing each clade. The tree is rooted to *Leucoagaricus barssii* (AFTOL ID 1899) and *L. meleagris* (30-VII-1996 Vellinga). Collections sequenced in the present study are highlighted in boldface. Black stars represent holotype specimens. ***

indicates taxon names modified according to Vizzini *et al.* (2011), Souza *et al.* (2022), and the present study. iNaturalist/Mushroom Observer photographs: **A**−**C**. ©Alejandro Tux, **D**. ©Alan Rockefeller, **E**. ©Harte Singer, **F**−**G**. © Renée Lebeuf, **H**−**I**. ©Stephen Russell.

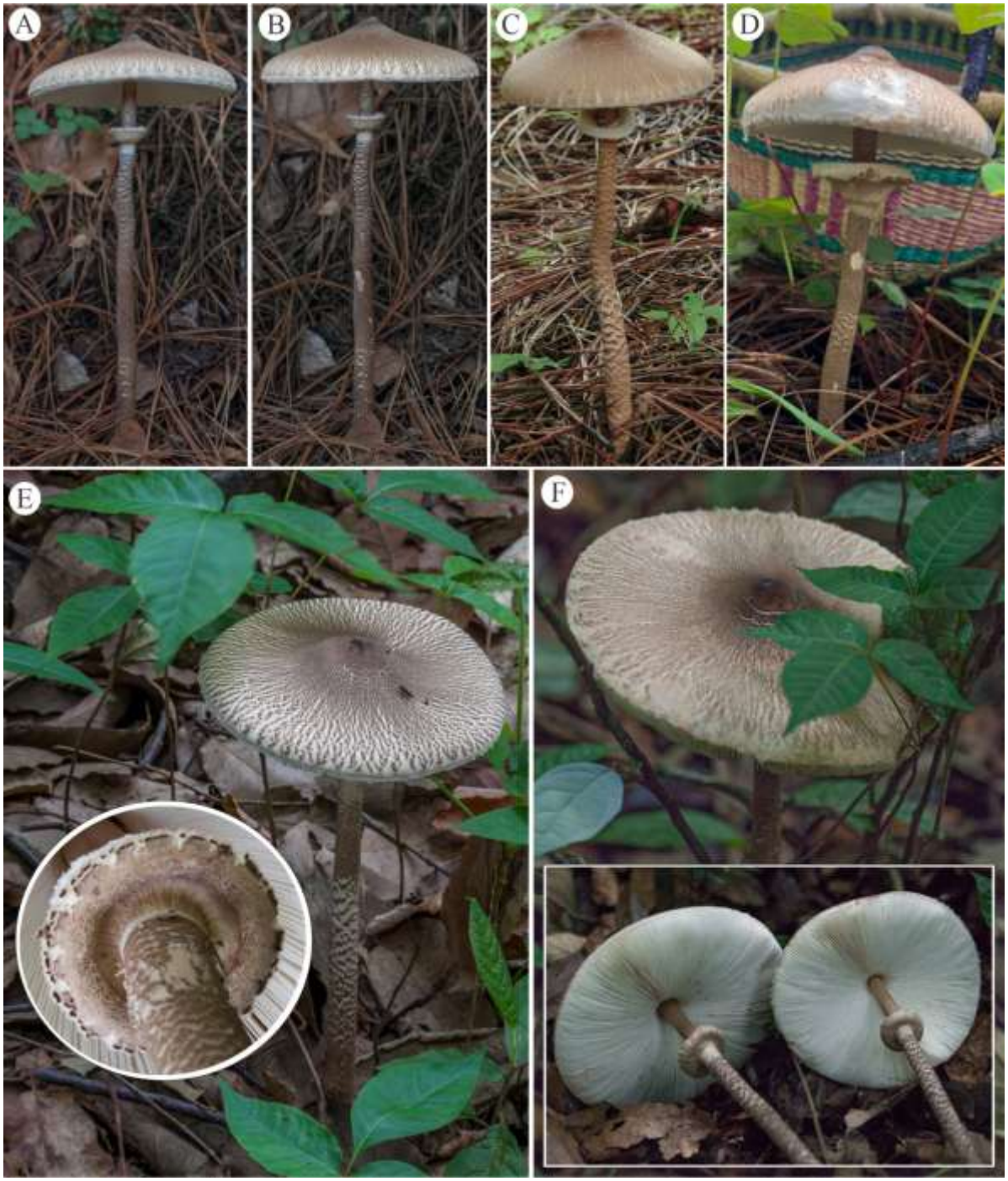

Figure 4. iNaturalist records phenotypically corresponding to the collection MO 306986 / iNat #194406677 (**F**) from Mexico. **A**–**B**, **E**. Tepoztlán. **C**–**D** Valle de Bravo. Photographs: **A**–**B**, **E**–**F**. ©Alejandro Tux, **C**. ©Mauricio Hernández, **D**. ©Zachary Hunter.

Taxonomy

Based on a combination of morphological, ecological, and molecular phylogenetic data, we introduce three new species and two new varieties of *Macrolepiota*. Morphologically, differences between species in /colombiana clade (viz. *M. bonaerensis*, *M. colombiana*, and *M. pernuda*) are subtle and species delimitation requires close analyses of a combination of macroscopic and microscopic (mainly stipitipellis, cheilocystidia, and basidiospores) characters.

Macrolepiota bonaerensis (Speg.) Singer (Figs. 5−6)
MycoBank No: 000000

≡ *Agaricus bonaerensis* Speg., Anal. Soc. cient. argent. 9(6): [278] (1880).
= *Lepiota bonaerensis* (Speg.) Speg. [as 'bonaërensis'], in Saccardo, Syll. fung. (Abellini) 5: 28 (1887).
= *Mastocephalus bonaerensis* (Speg.) Kuntze [as 'bonariensis'], Revis. gen. pl. (Leipzig) 2(1): 859 (1891).
= *Lepiota excoriata* f. *bonaerensis* (Speg.) Rick, Lilloa 1: 318 (1937).
= *Macrolepiota kerandi* (Speg.) Singer, Lilloa 22: 417 (1951) [1949].
= Lepiota kerandi Speg., *Anal. Mus. nac. Hist. nat. B. Aires* 6: 83 (1898) [1899].

Basidiomata medium to large-sized. Pileus 50−120 mm wide, fleshy, ovoid to campanulate when young, later campanulate-convex to convex or plano-convex, applanate or with a low umbo, with margin usually exceeding lamellae; surface dry, at first yellowish white (1A2) to cream (4A3), disrupting except at the disk into squamular patches on a white background; squamular patches small, brownish grey (5C2, 6C2) to greyish orange (6B3-4) or reddish grey, concentrically arranged, usually with recurving edges on all sides, bigger and paler towards margin, uncommon or absent in marginal zone; background usually fibrillose to coarsely fibrillose-ragged, sometimes showing a white context in between the fibrils; margin decurved and appendiculate. **Lamellae** free, remote, crowded, ventricose, up to 15 mm broad, white (1A1) to yellowish white (1A2), cream-beige (4A3, 5A3) and plentiful sordid vinaceous stains with age; lamellulae truncate, of several ranks, unevenly distributed; edge entire and concolor. **Stipe** 100−240 × 10−15 mm, cylindrical and slightly widening downwards, with a clavate or bulbous base (20−25 mm wide), sometimes with a white tomentose layer at base; surface whitish orange (6A2) to greyish orange (5B3, 6B3) or pale brownish grey (6C2), palest at the apex, farinose-furfuraceous to locally rimulose, rarely with adnate zig-zag bands that if present are always inconspicuous. **Annulus** superior, movable with age, complex, double;

upperside white and fringed; underside smooth and concolor with stipe surface. **Context** in pileus white (1A1) to white-cream (4A2), unchanging, thick (up to 13 mm) and dull; in stipe white and hollow with a central white cottony strand, unchanging. **Odor** none, indistinct or fungoid. **Taste** nutty and pleasant. **Spore print** white.

Figure 5. Basidiomata of *M. bonaerensis*. **A**. NCC290 (**FIFUNGI 1140**). **B**–**E**. NCC269 (**FIFUNGI 1141**). **F**. Pileus surface detail (NCC269) **G**. Annulus detail (Fazolino 362). Scale bars: 10 mm. Photographs: **A**. C. Coelho-Nascimento. **B**–**F**. J.M. Timm. **G**. Eduardo Fazolino Perez.

Basidiospores [400/16/16] (10.0−)11.0−16.2(−16.7) × (7.0−)7.3−9.3(−10.6) µm [$\mathbf{L_m}$= 13.0 µm; $\mathbf{W_m}$ = 8.2 µm; Q = 1.4−1.75(−1.8); $\mathbf{Q_m}$ = 1.56], ellipsoid to elongate, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous and metachromatic in Cresyl blue; apiculus about 1 µm long. **Basidia** (34−)37−52(−56) × 10.5−17.0 µm, clavate, pedunculate, hyaline, thin-walled, 4-spored. **Lamella edge** sterile, with crowded cheilocystidia. **Cheilocystidia** 17−43(−45) × 8.0−18.0(−20) µm, hyaline, colorless, thin-walled, variously shaped, mostly clavate to short clavate but also fusiform, pyriform or irregularly shaped, often catenulate. **Pleurocystidia** absent. **Pileus covering** an intricate trichoderm composed of chains that are 5–7 cells in length, fairly branching; intercalary elements (12.0−)15.0−65(−70) × (5.0−)7.0−20 µm, cylindrical, oblong or irregular shaped, occasionally with secondary septa, slightly thick-walled, with parietal, sometimes with intracellular brownish pigment, especially towards the upperside, often with an encrusting pigment in the basal elements; terminal elements very variable in shape and size, 15.0−80(−88) × 8.0−13.0(−15.0) µm, mostly cylindrical, clavate or lageniform but sometimes ovoid, fusiform or obtusely conic, distinctly thick-walled. **Stipe covering** a hymeniderm, colorless or with a diffuse yellowish pigment, composed of thin- to slightly thick-walled elements, predominantly clavate to broadly clavate, ventricose or inflated-fusiform but sometimes mucronate or with a short rostrum at the apex, 13.7−35.9 × 6.2−11.0 µm., less frequently slender clavate, lanceolate or cylindrical, 15.0−38 × 4.7−6.9 µm. **Clamp connections** common in the pileus context, stipe hyphae, and base of basidia.

Specimens examined: Brazil, Mato Grosso do Sul, Corumbá, Base de Estudos do Pantanal (BEP), 05 July 2019, *M.P. Drewinski* ***MPD477*** (FIFUNGI 1142), GenBank [ITS]: 00000000[4], [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000. Minas Gerais, Camanducaia, Serra da Mantiqueira, Sítio Mandaçaia, 14 December 2023, *C. Coelho-Nascimento & T.V.D.H. Comenale* ***NCC288*** (FIFUNGI 1138), GenBank [ITS]: 00000000, [nrLSU]: 00000000. São Paulo, São Paulo city, Parelheiros district, 20 January 2022, *C. Coelho-Nascimento* ***NCC183*** (FIFUNGI 1144), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 06 February 2024, *C. Coelho-Nascimento* ***NCC289*** (FIFUNGI 1139), GenBank [ITS]: 00000000, [nrLSU]: 00000000; *ibid. C. Coelho-Nascimento* ***NCC290*** (FIFUNGI1140), GenBank [ITS]: 00000000, [nrLSU]: 00000000. Rio Grande do Sul, Estância Velha, Bairro Industrial, 28 February 2023, *C. Coelho-Nascimento & J.M. Timm* ***NCC269*** (FIFUNGI 1141), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]:

[4] No momento da submissão desta tese, as sequências de DNA geradas neste estudo ainda não foram depositadas no GenBank. Os respectivos números de acesso serão disponibilizados após a publicação dos resultados.

00000000; *ibid.* 03 March 2023, *C. Coelho-Nascimento & J.M. Timm* ***NCC270*** (FIFUNGI 1143), GenBank [ITS]: 00000000, [nrLSU]: 00000000; Rio Grande, Estação Ecológica do Taim, 12 April 2016, *E. Fazolino Perez* ***000643*** **(**ICN 199621); *ibid.*, 02 March 2014, *E. Fazolino Perez* ***000148*** (ICN 199585); Canoas, Força Aérea Brasileira - Comando Aéreo Sul, 22 May 2014, *E. Fazolino Perez* ***000362*** **(**ICN 199583). Santa Catarina, Florianópolis, Morro da Lagoa da Conceição, trilha do jipe, 21 April 2011, *M.A. Neves, M. Jaeger; C. Leal-Dutra & J. Doria* ***752*** (FLOR 47985), GenBank [ITS]: 00000000; *ibid.* 13 April 2012, *A.C. Magnago, S. Urrea-Valencia, M. Jaeger & J. Simon-Cardoso* ***330*** (FLOR 47958), GenBank [ITS]: 00000000; Florianópolis, Câmpus da Universidade Federal de Santa Catarina, 15 April 2011**,** *E.R. Drechsler-Santos & M.A. Neves* ***574*** (FLOR 48051), GenBank [ITS]: 00000000; Urubici, Hotel Plaza Caldas da Imperatriz, Trilha da Cachoeira, 15 February 2013, *C. Heisecke* ***CHC54*** (FLOR 56090), GenBank [ITS]: 00000000; Urubici, Parque Nacional São Joaquim, Morro da Igreja, Pousada Véu de Noiva, 15 February 2013, ***CHC55*** (FLOR 56091), GenBank [ITS]: 00000000. Paraguay, Luque, Ñu Guazú Park, 15 June 2018 *M. Campi* ***MC294*** (FACEN5640), GenBank [ITS]: 00000000.

Habit, habitat, and distribution: solitary or gregarious, mostly in big groups, saprotrophic and terrestrial in meadows, pastures, parks, and along sideroads. Known from Argentina (Spegazzini 1880, Singer 1951, Singer & Digilio 1951, Wright & Albertó 2002), Brazil (Rick 1937, Singer 1953, Pegler 1997, Meijer 2006, Rosa & Capelari 2009, Putzke *et al*. 2014, this work), and Chile (Lazo 2016, Salazar-Vidal *et al*. 2017).

Notes − *Macrolepiota bonaerensis* is very easy to recognize in the field because of its white, fibrillose-ragged pileus background, the complex fringed annulus, and the common occurrence in grassland habitats (Singer & Digilio 1951). It is plausible that this species is experiencing an increase in numbers of population in Midwestern, Southeastern, and Southern Brazil due to the ongoing expansion and development of man-made pastures for cattle farming (Parente *et al*. 2019, Nehring 2023). Although most *M. bonaerensis* collections exhibit a fibrillose pileus surface and a relatively short stipe (not exceeding 1.5 × the pileus diam.), two collections (NCC183, NCC270) with a smooth pileus background and a very long stipe (around 2.5 × the pileus diam.) were found. These collections do not differ in any other character from the typical ones; therefore, no taxonomical importance was attached to these features likely caused by ecological circumstances.

The collections herein examined fit perfectly the morphological aspects with those of the protologue (Spegazzini 1880) and subsequent type studies (Singer 1951, Singer & Digilio

1951) of *M. bonaerensis*, mainly regarding the pileus covering, basidiospores dimensions, and ecology; differing only by the occasional longer stipe (100−240 mm). Prior to this study, *M. bonaerensis* had been overlooked and misidentified as *M. excoriata* [Rick 1907, 1939, 1961 as *Lepiota excoriata* (Schaeff.) P. Kumm., Raithelhuber 1988, 1991], *M. fuligineosquarrosa* Malençon (Alves *et al*. 2016), *M. mastoidea* [Raithelhuber 1988, 1991 as *M. gracilenta* var. *acuteumbonato* Raithelh., Putzke *et al*. 2014, Alves *et al*. 2016 as *M. gracilenta* (Krombh.) Wasser], and *M. procera* (Alves *et al*. 2016, Barroetaveña *et al*. 2016, Salazar-Vidal *et al*. **2017**), probably due to the somewhat pileus covering resemblance to these species.

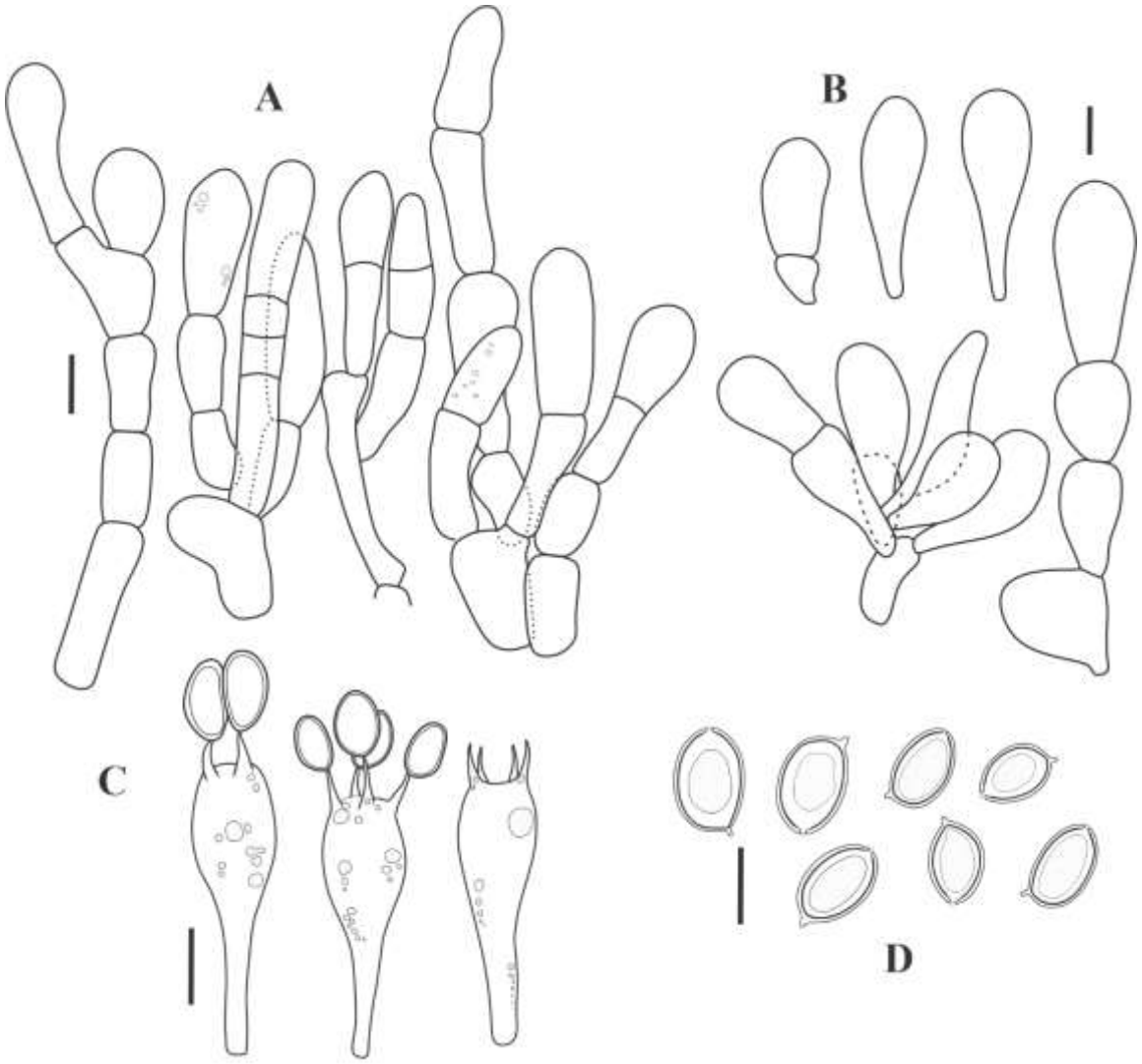

Figure 6. *Macrolepiota bonaerensis* (**NCC289**, **FIFUNGI 1139**). **A**. Pileus covering elements. **B**. Cheilocystidia. **C**. Basidia. **D**. Basidiospores. Scale bars = 10 μm. Line drawings: Cristiano Coelho-Nascimento.

Macrolepiota kerandi (Speg.) Singer is a rather poorly known species from South America that was originally described from Argentina as *Lepiota kerandi* Speg., growing under

arboreal canopies in open vegetation (Spegazzini 1898). It was described as a species that is closely related to *M. bonaerensis*, which was regarded as a far more commonly occurring species (Singer & Digilio 1951). Based on Spegazzini's texts (Spegazzini 1880, Spegazzini 1898), one may consider that the protologues of *M. kerandi* and *M. bonaerensis* concern different aspects of the same species. However, Singer (1951), Singer & Digilio (1951), and later works (Wright & Albertó 2002, Fazolino Perez *et al*. 2018, Maggio *et al*. 2021) kept them separated, still considering the shorther stipe, the more conspicuous pileus squamules, and the presence of cream tones in lamellae of *M. kerandi* as important to distinguish it from *M. bonaerensis* (Singer & Digilio 1951). In this sense, the species concept of *M. kerandi* may have been misconceived due the attachment of importance to non-reliable taxonomic traits, and the fact that *M. bonaerensis* shows a broad variation in macromorphology. The here revised type material of *M. kerandi* at LPS was found to have suffered considerable deterioration, however, the similar cheilocystidia, basidiospores, and the common presence of clamp connections observed in this original collection clearly supports our point of view. Therefore, we formally consider *M. kerandi* conspecific with *M. bonaerensis*.

Macrolepiota bonaerensis grouped within a strongly supported clade (BPP = 1.00, BS = 93 %) along with *M. colombiana*, *M. pernuda*, and a single unnamed collection from Mexico (MO 306986) (Figure 2). *Macrolepiota colombiana* can be distinguished from *M. bonaerensis* based on its distinctive tobacco-brown (6F6) stipe surface, which features adnate zigzag bands or squamulose girdles throughout (Franco-Molano 1999), contrasting with the paler, smooth to farinose-furfuraceous surface observed in the *M. bonaerensis*. Furthermore, *M. colombiana* exhibits a grayish-red context reaction (Franco-Molano 1999). From a microscopical perspective, *M. colombiana* produces rather thin-walled pileus covering elements, larger and less variable cheilocystidia (17−55 × 8−14 µm), and smaller basidiospores (12−14 × 7−10 µm) (Franco-Molano 1999). Furthermore, *M. colombiana* deviates from *M. bonaerensis* in the ecology; although the first also occurs is in grassy areas, it includes several collections from northern localities of South America in mixed forest dominated with *Quercus humboldtii* and species of *Pinus* and *Eucalyptus* (Franco-Molano 1999), besides is yet to be molecularly confirmed in other Neotropical areas. Differences of *M. bonaerensis* from the *M. pernuda* are discussed below under the latter species notes.

Macrolepiota capelariae* var. *capelariae A.D. Souza, C.C. Nascimento & Menolli, Phytotaxa 576(3): 270. (Fig. 7)

Holotype: Brazil, São Paulo, São Paulo city, Parque Estadual das Fontes do Ipiranga, 14 January 2022, *D.A. Zabin & M.C.S. Pires* ***DAZ060*** (SP513052, holotype), GenBank [*rpb2*]: 00000000. [*tef1-α*]: 0000000.

Taxonomic description: see Souza *et al*. (2022).

Additional specimens examined: Brazil, Minas Gerais, Camanducaia, Serra da Mantiqueira, Sítio Mandaçaia, 23 January 2022, *C. Coelho-Nascimento & T.V.D.H. Comenale* ***NCC184*** (FIFUNGI 1145), GenBank [ITS]: 00000000. Santa Catarina, Florianópolis, Rio Vermelho, 6 March 1986, *J. Furlani & C.L. Leite* (FLOR 10283**)**; Florianópolis, Universidade Federal de Santa Catarina**,** 5 October 2012**,** *C. Heisecke* ***CHC12*** (FLOR 56089), GenBank [ITS]: 00000000. São Paulo, São Paulo city, Marsilac district, Trilha Cachoeira dos Manacás, 27 January 2022, *C. Coelho-Nascimento* ***NCC185*** (FIFUNGI 1146), GenBank [ITS]: 00000000; Parelheiros district, Mata do Jardim dos Eucaliptos, 06 February 2024, *C. Coelho-Nascimento* ***NCC291*** (FIFUNGI 1153), GenBank [ITS]: 00000000; Parque Estadual das Fontes do Ipiranga (PEFI), 26 October 2022, *D.A. Zabin* ***DAZ172*** (FIFUNGI 1150); *ibid.* 24 July 2010, *M. Capelari, P.O. Ventura & N. Menolli Jr.* ***MC4584*** (SP513053)*,* GenBank [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 06 December 2010, *M. Capelari* ***MC4588*** (SP513054), GenBank [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 15 October 2023, *A.C. Morais & A.L. Vardiero* ***MAC55*** (FIFUNGI 1151); Limeira, Bairro dos Pires, Estrada Loiola, 7 January 2023, *D.A. Zabin* ***DAZ195*** (FIFUNGI 1152), GenBank [ITS]: 00000000; *ibid.* 28 Chácara Vale Verde, November 2023, *D.A. Zabin* ***DAZ276*** (FIFUNGI 1154); Marília, Bosque Municipal Rangel Pietraroia, 23 April 2024, *M.P. Drewinski & N. Menolli Jr.* ***MPD726*** (FIFUNGI 1155), GenBank [ITS]: 00000000; Rio Grande do Sul, São Francisco de Paula, bairro Remanso Indianópolis, Parador Hampel, 1 May 2023, *J.M. Timm* ***NCC285*** (FIFUNGI 1148), GenBank [ITS]: 00000000; Cambará do Sul, Parque Nacional de Aparados da Serra, Trilha do Itambézinho, 26 May 2021, T. *Kossmann, M. Titton, E.R. Drechsler-Santos, G.A. Alves-Silva & E.L. Gumboski* ***MIND.Funga0788*** (FLOR 71548), GenBank [ITS]: 00000000. Rio de Janeiro, Paraty, Fazenda Sobradinho, Trilha das Cavernas, 13 March 2024, *M.P. Drewinski* ***FB187*** (FIFUNGI 1080), GenBank [ITS]: 00000000.

Notes − *Macrolepiota capelariae* was recently described from Brazil with extended records to Argentina and Mexico. Putatively, this species occurs in several Brazilian states according iNaturalist (https://www.inaturalist.org.https://mushroomobserver.org) reports (Souza *et al*. 2022). Here, we formally first record *M. capelariae* to the states of Minas Gerais, Rio de

Janeiro, Santa Catarina, and Rio Grande do Sul. It is worth mentioning that the specimens from Rio Grande do Sul were found in association with Mixed Ombrophilous Forest and exhibit distinctly robust basidiomata (maximum pileus diameter: 200 mm; maximum stipe dimensions: 380 × 20 mm), a notable contrast to the collections studied by Souza *et al*. (2021), which were found solely in Dense Ombrophilous Forest and present smaller basidiomata (maximum pileus diameter: 120 mm; maximum stipe dimensions: 320 × 18 mm).

Figure 7. Basidiomata of *M. capelariae* var. *capelariae*. **A**, **B**. DAZ172 (**FIFUNGI 1150**). **C**−**E**. DAZ195 (**FIFUNGI 1152**). **F**−**H**. NCC285 (**FIFUNGI 1148**). Scale bars: 20 mm. Photographs: **A**–**E**. Denis A. Zabin. **F**–**H**. Jeferson M. Timm.

Macrolepiota capelariae* var**. ***velana C. Coelho-Nascimento & Menolli, **var. nov.**
(Figs. 8−9)
MycoBank No: 000000[5]

Diagnosis: *Macrolepiota capelariae* var. *velana* differs from the autonymous variety *M. capelariae* var. *capelariae* by the pileus surface with conspicuous deep furrows/grooves (80 × 30 mm, radially oriented, from margin to near the center) and whitish velar patches, the mostly catenulate cheilocystidia (short clavate, clavate to fusiform or oblong), larger basidiospores (14.8−20.2 × 9.8−11.7 μm), and the almond-like odor.

Etymology: *veli* (L.) = veil; referring to the presence of velar remnants on the pileus surface.

Holotype: Brazil, São Paulo, Ubatuba, Parque Estadual da Serra do Mar, Núcleo Picinguaba, Trilha dos Poços, 16 February 2022, *D.A. Zabin, M.P. Corrêa-Santos & C. Coelho-Nascimento* ***DAZ105*** (FIFUNGI 1149), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Basidiomata large-sized. **Pileus** 140−200 mm wide, fleshy, ovoid to conic-ovoid when young, expanding to umbonate with a high umbo, straight margin; surface dry, with conspicuous deep furrows/grooves (80 × 30 mm, radially oriented, from margin to near the center), at first completely pale greyish brown ($Y_{20}M_{10}C_{10}$−$Y_{30}M_{20}C_{10}$) to pale yellowish brown ($N_{20}Y_{40\text{-}50}M_{20}$), later finely breaking up into concolor radially arranged interwoven strips or into small appressed-fibrillose squamules, barely exposing the background; background glabrous, white to yellowish white ($Y_{10}M_{00}C_{00}$); central umbo grayish orange ($Y_{70}M_{40}C_{10}$) to pale orange-brown ($Y_{80}M_{40}C_{10\text{-}20}$), with white to off-white membranous velar remnants as small patches. **Lamellae** free, crowded, ventricose to broad, up to 14 mm broad, white; lamellulae subtruncate, of several ranks, unevenly distributed, edge entire, smooth, concolor. **Stipe** 300−420 × 15−20 mm, protruding into the pileus, central, cylindrical with slight wider downward base, with a small basal bulb (18−22 mm wide) that is completely covered by a tomentose-velvety white

[5] Os números de registro no MycoBank referentes a todas as novas espécies propostas nesta tese serão atribuídos apenas quando da publicação dos respectivos artigos taxonômicos.

mycelial layer, with long white rhizomorphs; surface glabrous, longitudinally fibrous striate, pale greyish brown ($Y_{20}M_{10}C_{10}$–$Y_{40}M_{20}C_{20}$) to pale brown ($Y_{50}M_{40}C_{30}$). **Annulus** superior, descending, membranous, smooth, persistent but easily removable, whitish with a hazel edge. **Context** in the pileus white and thick, in stipe white and hollow with a central white cottony strand, unchanging in both. **Odor** almond-like. **Taste** very pleasant, a somewhat sweet, earthy and nutty. **Spore print** white.

Figure 8. Basidiomata of *M. capelariae* var. *velana* (DAZ105, **FIFUNGI 1149**, holotype). Scale bars: 20 mm. Photographs: Cristiano Coelho-Nascimento.

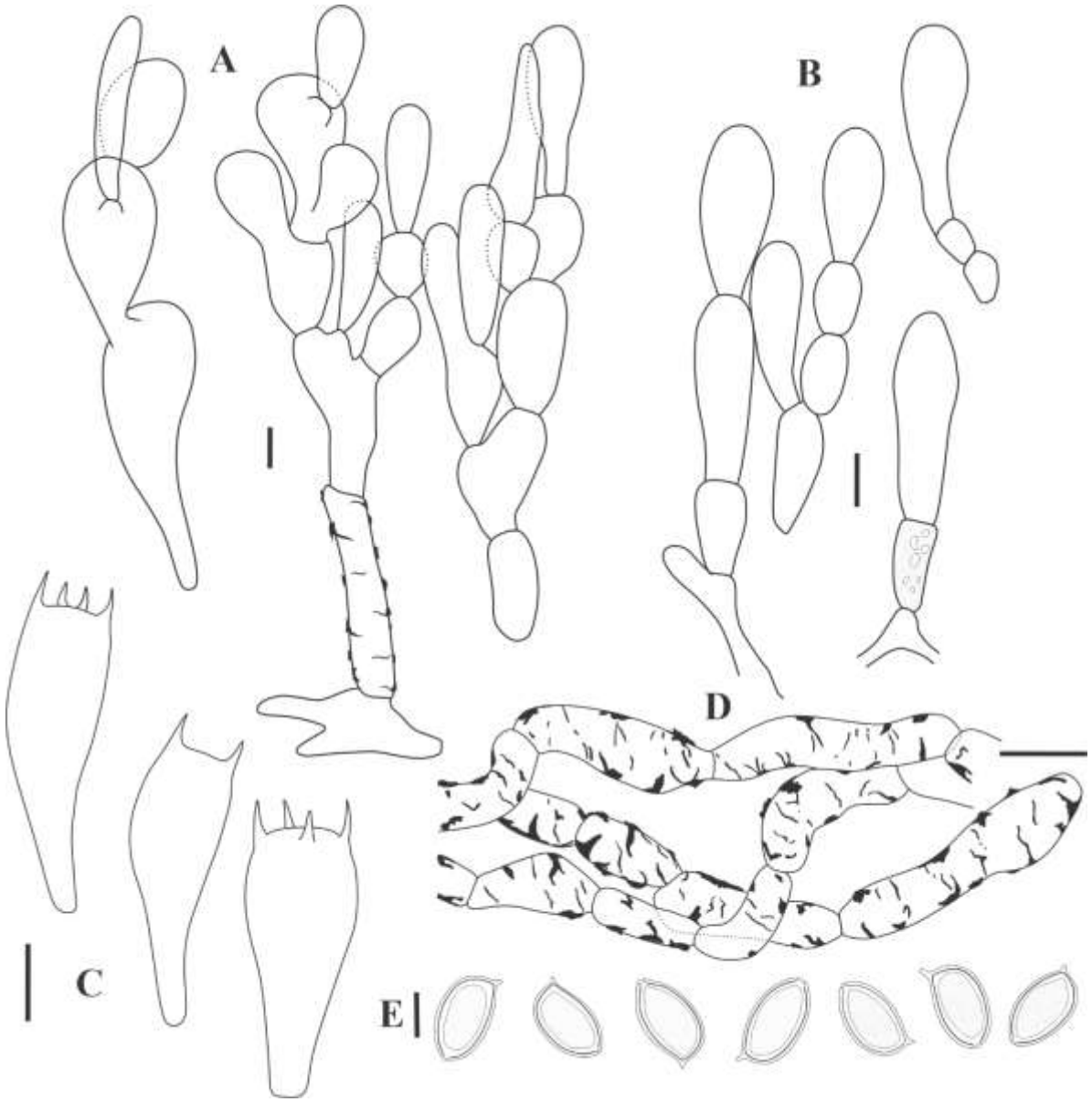

Figure 9. *Macrolepiota capelariae* var. *velana* (DAZ105, **FIFUNGI 1149**, holotype). **A**. Pileus covering elements. **B**. Cheilocystidia. **C**. Basidia. **D**. Velar patches elements. **E**. Basidiospores. Scale bars = 10 µm. Line drawings: Cristiano Coelho-Nascimento.

Basidiospores [90/3/2] (11.8−)14.8−20.2(−21) × (7.8−)9.8−11.7(−12.0) µm (**L_m** = 18.6 µm; **W_m** = 11.2 µm; Q = 1.62−1.68; **Q_m** = 1.65), elongate, sometimes ellipsoid, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous and metachromatic in Cresyl blue; apiculus about 1.2 µm long. **Basidia** 20−48(−52) × 10.0−18.0 µm, clavate, hyaline, thin-walled, mostly 4-spored. *Lamella edge* sterile, with crowded cheilocystidia. **Cheilocystidia** 19−68 × 8.0−18.0(−20) µm, hyaline, colorless, thin-walled,

short clavate, clavate to fusiform or oblong, rarely pyriform, mostly catenulate, in bunches forming a sterile edge. **Pleurocystidia** absent. **Pileus covering** a trichoderm composed of chains that are 4–6 cells in length, distinctly branching; lower layer elements subglobose, broadly clavate to oblong or cylindrical, (15.9–)15.5–87.7 × (10.2–)11.9–33.6 µm, slightly thick- to thick-walled, with pale yellowish brown intracellular pigments, walls sometimes slightly encrusted; upper layer elements clavate, narrowly clavate, lageniform to acuminate (apically always obtuse or constricted), (65.4–)61–32.9(–26.6) × 12.3–16.7(–18.0) µm, slightly thick- to thick-walled, colorless or with pale yellowish brown parietal pigment; trichoderm arising from an underpinning layer composed of loosely interwoven hyphae, smooth to slightly incrusted, thin-walled, sometimes partially inflated, 4.0–18.0 µm diam. **Velar patches** composed of elongate, cylindrical hyphae, thin-walled, 2.5–6.0 µm diam., branched, sometimes anastomosed, intermixed with partially inflated hyphae that are oblong to allantoid, slightly thick- to thick-walled, bearing conspicuous bands or fragments of encrusting pigment, 9–15 µm diam. **Stipe covering** a hymeniderm of cylindrical elements, 10.0–18.5 µm diam., thin-walled, with a diffuse pale brownish pigment. **Clamp connections** rare, only seen at the stipe context hyphae.

Additional specimen examined: Brazil, São Paulo, Ubatuba, Parque Estadual da Serra do Mar, Núcleo Picinguaba, Trilha Jatobá, 25 February 2022, *C. Coelho-Nascimento, D.A. Zabin & M.P. Corrêa-Santos* ***NCC202*** (FIFUNGI 1147), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Habit, habitat, and distribution: saprotrophic, terrestrial, growing solitary or scattered among herb assemblages on the outskirts of a Dense Ombrophilous Forest fragment. Only known for the Brazilian state of São Paulo, Southeastern Brazil.

Notes – *Macrolepiota capelariae* is a common species with a broad Neotropical distribution (Souza *et al*. 2022). In light of the additional records (under *M. capelariae* var. *capelariae* examined collections) and more precise monitoring of *Macrolepiota* entries within the iNaturalist database for the Neotropics, it is now possible to recognize the typical *M. capelariae* phenotype pattern, which appears to be conserved across the species distribution, from Mexico to Argentina. Notably, two recent *Macrolepiota* collections (DAZ105 and NCC202), found in a Dense Ombrophilous Forest fragment in Southeastern Brazil, immediately evoked the *M. capelariae* general aspect in the field, albeit with some clearcut distinctions. Further molecular examination confirmed the conspecificity with *M. capelariae*, but the striking combination of

morphological, anatomical, and organoleptic features makes these collections unique under the *M. capelariae* concept, what supported the introduction of a new varietal rank. The typical variety of *M. capelariae* is recognized by the combination of light brown, pale greyish brown to brownish orange or clay brown pileus surface, mostly breaking up into radially arranged interwoven strips on white background, the simple annulus, the narrowly clavate cheilocystidia that are often catenulate (21−50 × 9.0−15.0 µm), and the ellipsoid to elongate basidiospores (12.5−15.0 × 7.5−11.2 µm) (Souza *et. al*. 2022), and the mild fungoid odor/taste. In turn, the local phenotype *M. capelariae* var. *velana* displays a distinctly grooved pileus surface with white velar patches, a sweet nutty taste, an almond-like odor, larger cheilocystidia (19.0−68 × 8.0−18.0 µm) that are mostly catenulate, and larger basidiospores (14.8−20.2 × 9.8−11.7 µm). It is also worth mentioning that in the *M. capelariae* var. *velana* collections examined, the veil remnants on the pileus (mainly at umbo) were observed in both young and mature basidiomata, representing a consistent phenological feature associated with the newly proposed variety.

Macrolepiota chapeleta C. Coelho-Nascimento & Menolli, **sp. nov.** (Figs. 10−11)
MycoBank No: 000000

= *Lepiota procera* var. *vulpina* Rick, Lilloa 1: 317 (1939) [1937].

Diagnosis: *Macrolepiota chapeleta* is recognized by the large, light orange, golden yellow to grayish orange pileus, white to yellowish white lamellae, yellowish orange to brownish yellow-orange stipe with a simple membranous annulus, mostly elongate basidiospores (13.6–27.2 × 13.4–26.2 µm), clavate, broadly clavate to ventricose cheilocystidia (25−56 × 12−15 µm), and the irregular trichodermal pileus covering composed of mostly globose, subglobose to ellipsoid elements.

Etymology: the epithet '*chapeleta*' refers to the vernacular name assigned to wild edible *Macrolepiota* specimens by some local citizens and means, in Portuguese, a small hat with a narrow brim.

Holotype: Brazil, São Paulo, São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, 13 January 2022, *C.C. Nascimento, D.A. Zabin & A.C. Morais* ***NCC155*** (FIFUNGI 1129), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Basidiomata medium to large-sized. **Pileus** 80−260 mm wide, fleshy, ovoid to subglobose or hemispherical when young, expanding to convex or plano-convex, with a low

umbo at disc; surface dry, often speckled with dark punctations/streaks, covered with light orange ($Y_{60}M_{20\text{-}30}C_{00}$), golden yellow ($Y_{80}M_{30\text{-}40}C_{10}$−$Y_{90}M_{40\text{-}50}C_{10}$) to grayish orange ($Y_{80}M_{40}C_{20}$) appressed squamules, rarely breaking up into small to large patches; squamules radially oriented, crowded, then more spread out at margin and slightly exposing a pale orange ($Y_{30}M_{10}C_{00}$) to yellowish orange ($Y_{40}M_{10}C_{00}$) background in between, disc smooth, dark brown ($N_{70}Y_{40\text{-}60}C_{40\text{-}50}$). **Lamellae** free, remote, crowded, ventricose, wider at pileus margin and narrower towards pileus center, up to 10 mm wide, white to yellowish white ($Y_{10}M_{00}C_{00}$); lamellulae truncate to subtruncate, plentiful, of several ranks, unevenly distributed; edge entire, smooth, concolor. **Stipe** 150−500 × 10−18 mm, central, cylindrical, slightly wider downward base, bulbous at base; bulb subglobose, 22−35 mm wide, completely covered by a tomentose-velvety white mycelial layer; upper half surface glabrous, waxy, light yellow ($N_{00}Y_{50\text{-}70}M_{10}$) to yellowish orange ($N_{00}Y_{50\text{-}80}M_{20}$); lower half surface appressed-fibrillose, brownish yellow ($N_{20}Y_{80\text{-}99}M_{40}$) to brownish orange ($N_{20}Y_{80\text{-}99}M_{50}$), sometimes forming adnate zigzag bands or adnate squamulose girdles. **Annulus** superior**,** descending, membranous, off-white to yellowish white ($Y_{20}M_{00}C_{00}$), with brownish patchy squamules on the underside, movable when mature. **Context** in the pileus thick, spongy, white, unchanging; in stipe fibrous, white, and hollow with a central white cottony strand, unchanging. **Odor** mild, fungoid. **Taste** mild or indistinct. **Spore print** white.

Basidiospores [240/8/8] (19.0−)20−26.3(−30.3) × (11.5−)12.1−14.5(−15.2) µm (**L_m**= 23.3 µm; **W_m** = 13.1 µm; Q = 1.55−2.08; **Q_m** = 1.78), elongate, rarely ellipsoid or cylindrical, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, and metachromatic in Cresyl blue; apiculus sublateral to subapical, very prominent to rather small; contents mono- or multi-guttulate. **Basidia** 40−75 × 20−25 µm, clavate to broadly clavate, pedunculate, hyaline, thin-walled, 4- or (occasionally) 2-sterigmate. **Lamella edge** sterile, with crowded cheilocystidia. **Cheilocystidia** 25−56 × 12.0−15.0(–17.0) µm, broadly clavate, clavate to ventricose, often catenulate, hyaline, colorless, thin-walled. **Pleurocystidia** absent. **Pileus covering** an irregular trichoderm composed of chains that are 4–9 cells in length, often branching; inflated elements slightly thick- to thick-walled, hyaline or with golden brown parietal and intracellular pigments, mostly globose, subglobose to ellipsoid (12.0–)13.6–27(–28) × (11.5–)13.4–26 µm, less frequently broadly clavate, clavate, oblong to elongate (26–)27–60 × (7.2–)11.2–30 µm; cylindrical to slender cylindrical (inflated to partially inflated) elements present at base, 34–55(–60) × 8.2–15.7(–16.3) µm; trichoderm arising from an underpinning layer composed of repent cylindrical hyphae, 3.0–16.0 µm diam., subregularly to irregularly arranged, heavily or moderately encrusted. **Stipe covering** a cutis, composed of

parallel, compactly arranged, thin-walled, cylindrical hyphae, 3.0–13.0 diam., hyaline or yellowish in KOH. **Clamp connections** rare, only seen at the stipe context hyphae.

Figure 10. Basidiomata of *M. chapeleta* (NCC155, **FIFUNGI 1129**, holotype). Scale bars: 10 mm. Photographs: Cristiano Coelho-Nascimento.

Additional specimens examined: Brazil, São Paulo, São Paulo city, Universidade de São Paulo (USP), Câmpus São Paulo, Parque Esporte para Todos, 24 March 2022, *C.C. Nascimento & D. Mendel* ***NCC203*** (FIFUNGI 1131), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; São Paulo city, Parelheiros district, Mata do Jardim dos Eucaliptos, 28 January 2022, *C.C. Nascimento* ***NCC187*** (FIFUNGI 1130), GenBank [ITS]: 00000000; *ibid.* 04 June 2024, *C.C. Nascimento* ***NCC294*** (FIFUNGI 1132), GenBank [ITS]: 00000000; Limeira, Bairro dos Pires, Chácara Vale Verde, 9 January 2023 *D.A. Zabin* ***DAZ200*** (FIFUNGI 1133), GenBank [ITS]: 00000000; Gália, Estação Ecológica dos Caetetus, 18 October 2023, *G. Mariano-Silva & N.M. Nitzsche* ***BDIF908*** (FIFUNGI 1134), GenBank [ITS]: 00000000; Paraguay, Caazapá, San Rafael National Park, 15 January 2021 *Y. Maubet* ***YM183*** (FACEN5641), GenBank [ITS]: 00000000, [nLSU]: 00000000; Itapúa, San Rafael National Park, 16 January 2021 *E. Cristaldo* ***EC051*** (FACEN5642), GenBank [ITS]: 00000000.

Habit, habitat, and distribution: solitary to gregarious, saprotrophic, and terrestrial on humus-rich soils in Dense or Mixed Ombrophilous Forest fragments, copses or city-parks. Known from Argentina (Fazolino Perez *et al*. 2018 as *Macrolepiota* sp.), Brazil and Paraguay (this work).

Notes: Although based on unnamed or misidentified previous records, this species has been long known in Brazil, mainly by their common occurrence and its prized culinary value. A review of online mushroom identification forums from South America on Facebook (viz., Hongos, identificación y discusión; Cogumelos do Brasil; Identificação de Fungos - Brasil; Hongos de América Latina - Mushrooms of Latin America; and Hongos y líquenes de América) over a three-year period (2021–2023) revealed that *M. colombiana* is the most common name assigned to records that putatively represent *M. chapeleta* described here, although both species present an easily distinguishable macroscopic appearance. *Macrolepiota colombiana* can be distinguished from *M. chapeleta* by the brown pileal squamules/patches and white fibrillose background, the more complex annulus (sometimes with a double edge), and the smaller and non-elongate basidiospores (12–14 × 7–10 µm) (Franco-Molano 1999). Phylogenetically, *M. chapeleta* sister to the clade containing '*M. capelariae* + *M. abruptibulbosa*' within the clade /capelariae (Figure 2). From a morphological perspective, *M. capelariae* can be separated from *M. chapeleta* in the smaller basidiomata (maximum pileus diameter: 120 mm; maximum stipe dimensions: 150 × 320 mm), the mostly narrowly clavate cheilocystidia, and the smaller and mostly ellipsoid basidiospores (12.5−20.2 × 7.5−11.7 µm) (Souza *et al*. 2022). The pileus surface is also diagnostic for segregating both species, although it should be cautiously

interpreted as it may vary with age and weather conditions. The pileus surface of *M. chapeleta* mostly exhibits orange tones with distinct black punctations/streaks and rarely breaks to expose the background, whilst in *M. capelariae* it mostly breaks into brownish radial strips, with the background remaining distinctly exposed with age (Souza *et al*. 2022). Differences between *M. chapeleta* and *M. abruptibulbosa* are discussed below under the latter species notes.

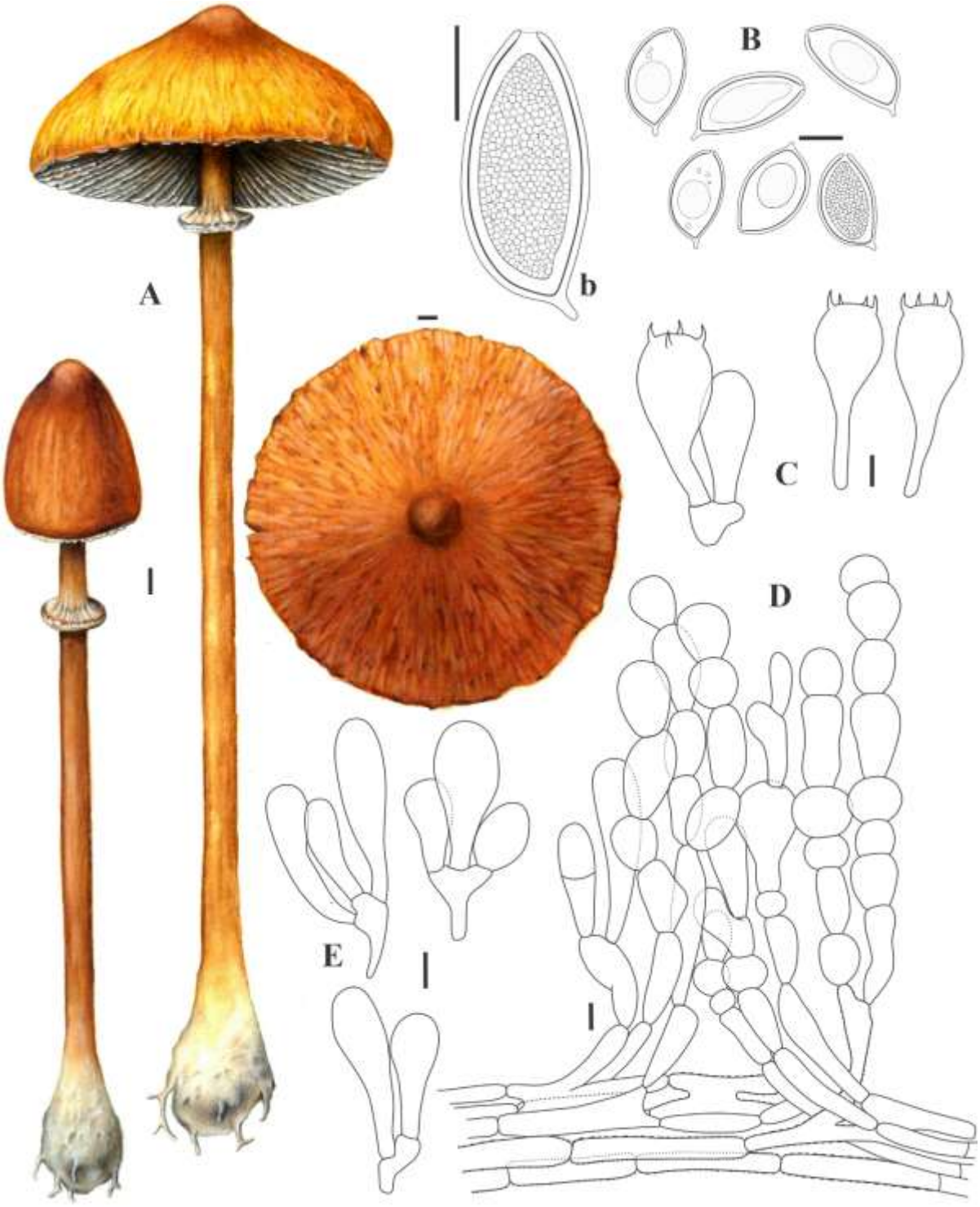

Figure 11. *Macrolepiota chapeleta* (NCC155, **FIFUNGI 1129**, holotype). **A**. Basidiomata. **B**/**b**. Basidiospores / detail of the multigutullate contents. **C**. Basidia. **D**. Pileus covering elements. **E**. Cheilocystidia. Scale bars: A = 10 mm; B−E = 10 µm. Watercolor illustrations: Dyego Costa. Line drawings: Cristiano Coelho-Nascimento.

The main morphological concept of *M. chapeleta* perfectly matches with *Lepiota procera* var. *vulpina* Rick, originally described from Southern Brazil (Rick 1939). As evidenced by Souza *et al*. (2022), no painting, drawing or collection were designated as holotype in Rick's protologue, and they could not trace any collection under this varietal name at PACA herbarium (curator, pers. comm.) neither at the database (https://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm) of the 'Lloyd Herbarium' collections that are now part of the U.S. National Fungus Collections (BPI), both where the Rick's collections are deposited. Despite that, Souza *et al*. (2022) considered assigning Rick's varietal name as a synonym when describing *M. capelariae* but cautiously refrained from this proposing as no concordant morphological aspects were recognized. *Lepiota procera* var. *vulpina* was described as a "vulpine-fulva" species with distinctly large basidiospores (14–22 µm), and except for *M. chapeleta*, no other commonly occurring *Macrolepiota* species from Southern Brazil (viz., *M. abruptibulbosa*, *M. bonaerensis*, *M. capelariae*, *M. cyanolamellata* and *M. pulchella*) could directly evoke these diagnostic traits. For this reason, we decided to formally include the Rick's varietal name as a synonym of the newly described species. The conviction to establish this synonymy is ultimately based on years of experience sampling *Macrolepiota* specimens in Southern Brazil, as no definitive confirmation can be made due to the unavailability of the type material and a lack of detailed morphological information for the aforementioned variety. Moreover, the proposal to designate *M. chapeleta* as a novel taxon, rather than considering the related varietal name, is consistent with the International Code of Nomenclature for algae, fungi, and plants, which stipulates that a name has no priority outside the rank at which it is published (Chapter II, Article 11.2) (Turland *et al*. 2018).

Based on records from GBIF and GenBank (MN847714 and MN847715), the distribution of *M. chapeleta* can be putatively extended to Argentina and to other two Brazilian states: Minas Gerais and Rio de Janeiro (Figure 12). Different from *M. capelariae* that occurs more commonly and has a wide Neotropical distribution, *M. chapeleta* putatively has a distribution limited to southern South America and does not appear to overlap with the range of *M. colombiana*, which seems to be restricted to northern South America (Souza et al. 2022). Indeed, a careful examination of all *Macrolepiota* observations from Colombia, Ecuador, and Venezuela at iNaturalist (totaling 290 records), we failed to identify any reports that could

resemble the general appearance of *M. chapeleta*, thereby supporting the assumption that its range is restricted to southern part of the continent. However, in the absence of a wide phylogenetic taxon sampling from northern South America, as well as from Central America and Mexico, it is virtually impossible to drawn reliable taxonomic conclusions on the ecological and distributional boundaries of *M. chapeleta* and *M. colombiana*.

Figure 12. Putative records of *M. chapeleta* retrieved from GBIF/iNaturalist. **A**−**B, D.** Brazil (**A**: Itatiaia, Rio de Janeiro state; **B**: Santo Antônio do Manhuaçu, Minas Gerais state; **D**: Conceição do Para, Minas Gerais state). **C**: Argentina (Puerto Iguazú). . Photographs: **A.** ©Graziele Noronha, **B.** ©Mariane Kaizer, **C.** ©Daniel Alejandro Paiz, **D.** ©Agnaldo Correa de Assis.

Macrolepiota pernuda C. Coelho-Nascimento & Menolli, **sp. nov.** (Figs. 13, 14)
MycoBank No: 000000

Diagnosis: *Macrolepiota pernuda* differs from *M. colombiana* in its larger basidiomata (maximum reported pileus diameter of 280 mm, maximum stipe dimensions are 300 × 10 mm), unchanging pileus context, simple and descending annulus, slightly bulbous stipe base, and mostly ellipsoid basidiospores.

Etymology: the specific epithet '*pernuda*' refers to the vernacular name assigned by some local citizens to wild edible *Macrolepiota* specimens, which means long legs in Portuguese, in reference to its conspicuously long stipe.

Holotype: Brazil, São Paulo, São Paulo city, Parque Estadual Campus do Jordão (PECJ), Trilha do Rio Sapucaí, 21 January 2020, *N. Menolli Jr.*, *M.P. Drewinski*, *M.F.O Silva*, *M.P.C Santos* & *T.R Santos* ***NMJ279*** (FIFUNGI 1160), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Figure 13. Basidiomata of *M. pernuda*. **A**−**B**, **D**−**E**, **G**. NMJ279 (**FIFUNGI 1160**, holotype). **C**, **F**. CT03. Scale bars: 10 mm. Photographs: **A**−**B**, **D**−**E**, **G**. Nelson Menolli Jr.**C**, **F**. Thiago V. D. H. Comenale.

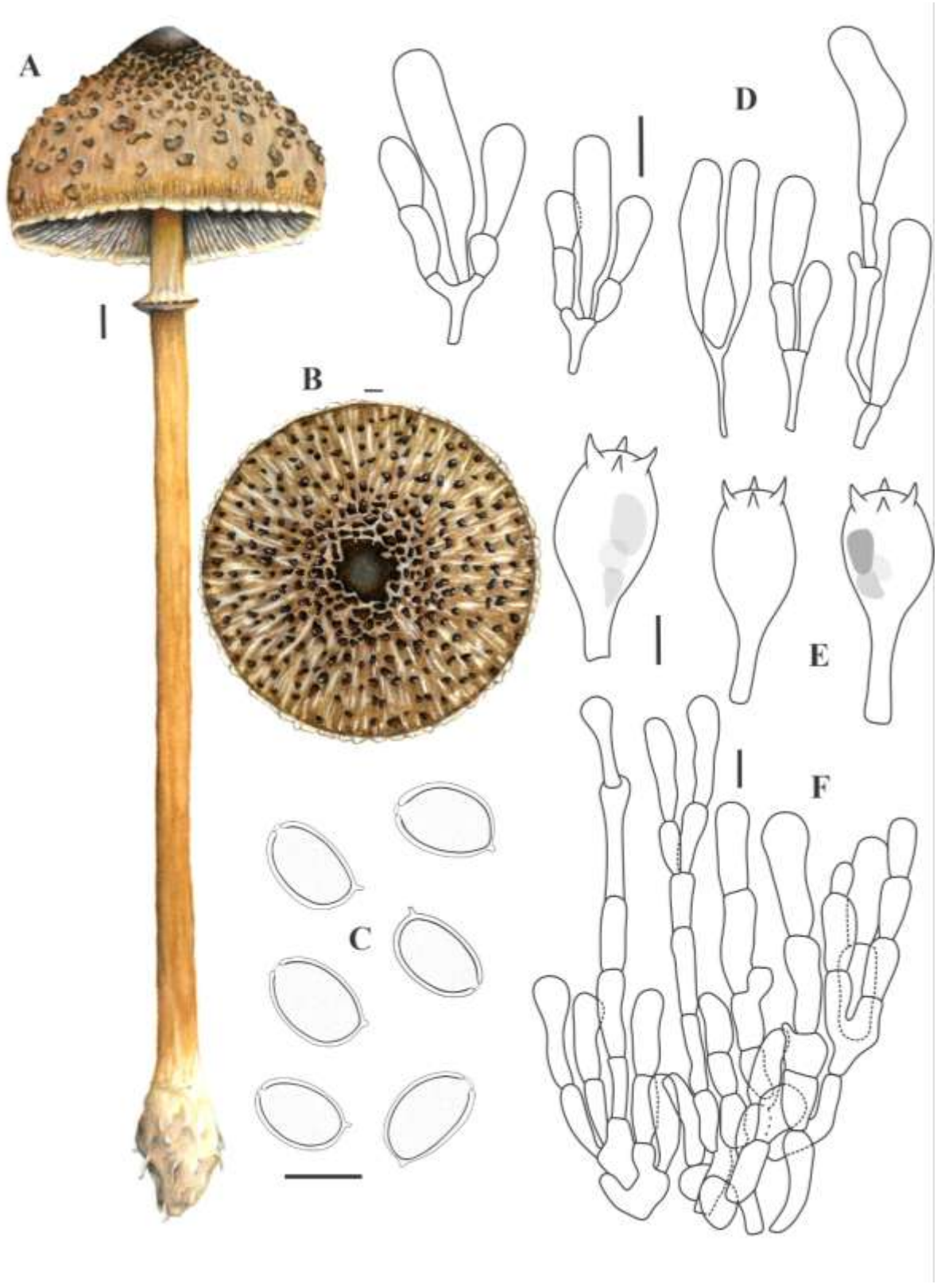

Figure 14. *Macrolepiota pernuda* (NMJ279, **FIFUNGI 1160**, holotype). **A**. Basidioma. **B**. Mature pileus in top view. **C**. Basidiospores. **D**. Cheilocystidia. **E**. Basidia. **F**. Pileus covering elements. Scale bars = **A**,**B** = 10 cm; C−F = 10 µm. Watercolor illustrations: Dyego Costa. Line drawings: Cristiano Coelho-Nascimento.

Basidiomata large-sized. **Pileus** 80−240 mm wide, fleshy, ovoid to obtusely conic when young, sometimes with a cracked surface, then expanding to campanulate and finally convex to plano-convex, umbonate; surface dry, light greyish brown ($Y_{40}M4_0C_{40}$) to greyish brown ($N_{80}Y_{50\text{-}60}M_{20}$), later breaking into a central calotte that is dark greyish brown ($N_{90}Y_{10\text{-}20}M_{10\text{-}20}$) to dark brown ($N_{90}Y_{40}M_{20}$, $N_{99}Y_{50}M_{40}$) and surrounded by small patch- or crust-like squamules (sometimes as fine pulverulence towards margin), not or slightly uplifted, on a radially appressed-fibrillose covering composed of greyish tan ($N_{20}Y_{40}M_{30}$), pale brown ($Y_{30}M_{30}C_{20}$) to wood brown ($Y_{60}M_{40}C_{20}$) strands on a white to beige background; margin straight to inflexed, sometimes appendiculate to fringed, white to yellowish white ($N_{00}Y_{10}M_{00}$). **Lamellae** free, remote, crowded, ventricose, up to 11 mm broad, white; lamellulae subtruncate, plentiful, of several ranks, unevenly distributed; edge minutely gnawed, with indentations shallow and irregular, concolor. **Stipe** 150−300 × 10−18 mm, protruding into the pileus, central, cylindrical to slightly tapering toward apex; base clavate or forming a bulb (up to 22 mm); surface pale brown ($Y_{30}M_{30}C_{20}$) to pale orangish brown ($Y_{40}M_{30}C_{10}$), paler upwards, sometimes breaking open into concolor zigzagging bands over the whole length. **Annulus** superior**,** simple**,** descending, membranous, thin, velvety, persistent but easily removable; upperside white to yellowish white ($N_{00}Y_{10}M_{00}$); underside concolor with upperside, often forming a brownish squamulose edge. **Context** white and thick in pileus, unchanging; white and hollow in stipe, with a central white cottony strand, unchanging. **Odor** and **taste** not observed. **Spore print** white.

Basidiospores [180/6/4] (9.6−)9.9−14.8(−15.3) × (7.0−)7.4−9.7(−10.0) µm [$\mathbf{L_m}$= 12.7 µm; $\mathbf{W_m}$ = 8.6 µm; Q = (1.29−)1.34−1.70(−1.76); $\mathbf{Q_m}$ = 1.48], mostly ellipsoid, sometimes elongate, rarely broadly ellipsoid or cylindrical, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, and metachromatic in Cresyl blue; apiculus sublateral to subapical, apex rounded or sometimes obtuse or truncate, contents granular or multi-guttulate. **Basidia** 32−40(−43) × 13.0−14.2(−15.0) µm, clavate to ventricose, often pedunculate, hyaline, thin-walled, 4-spored, rarely 2-spored. **Lamella edge** composed of a broad sterile zone of crowded cheilocystidia. **Cheilocystidia** 26−50(−63) × (6.6−)7.9−20(−22) µm, crowded**,** mostly clavate or slender clavate to short clavate, less frequently cylindrico-clavate, hyaline, colorless, thin-walled. **Pleurocystidia** absent. **Pileus covering** a trichoderm composed of erect, densely packed hyphae, 130–220 µm long, septate, thin- to slightly thick-walled (~0.5 µm wide); intercalary elements branching, with pale brownish yellow parietal and intracellular pigments, predominantly clavate, slender clavate to cylindrical, 15.0–33(−38) × 4.5–8.0 µm, less frequently oblong to somewhat irregular, 8.1–16.0(−18.0) × 7.6–11.8 µm; terminal elements cylindrical, apex obtuse, rarely tapered;

trichoderm arising from an underpinning layer composed of repent elongated cylindrical hyphae, 4.3–8.0 µm diam., infrequently branching, with pale yellowish-brown pigments, slightly thick-walled, encrusted as flakes. **Stipe covering** a trichoderm composed of erect to suberect hyphae, branching, (~150 µm long), with a diffuse yellowish brown pigment; terminal to subterminal elements predominantly tapering towards apex, sometimes with secondary septa, 26–66 × 3.6–11.0 µm; elements below predominantly cylindrical, less frequently oblong, short clavate or irregularly shaped, 12.0–26 × 2.5–8.6 µm. **Clamp connections** common at the base of basidia and in the stipe contextual hyphae.

Additional specimens examined: Brazil, Minas Gerais, Camanducaia, Serra da Mantiqueira, Sítio Mandaçaia, 19 February 2022, *C. Coelho-Nascimento & T.V.D.H. Comenale* ***NCC205*** (FIFUNGI 1158), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 24 May 2022, *T.V.D.H. Comenale* ***CT03*** (FIFUNGI 1161), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.; *ibid.* 19 February 2022, *C. Coelho-Nascimento* & *T.V.D.H. Comenale* ***NCC201*** (FIFUNGI 1159), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Habit, habitat, and distribution: solitary to scattered, saprotrophic, and terrestrial. Uncommon species, only found in high altitudes (ca. 1600 m a.s.l). Known from Mixed Ombrophilous Forest in the states of Minas Gerais and São Paulo, Southeastern Brazil.

Notes: As phylogenetically circumscribed here, *M. pernuda* constitutes a monophyletic assembly in the /colombiana clade (*M. colombiana* complex) (Figure 2) that is so far restricted to highlands Mixed Ombrophilous Forest (ca. 1600 m a.s.l) in Southeastern Brazil. *Macrolepiota pernuda* is molecularly close to *M. bonaerensis* and *M. colombiana*, from which it can be distinguished by 6 and 9 nucleotide differences, respectively, representing only 1.3−1.5% of sequence divergence at the ITS locus.

Macrolepiota pernuda and *M. bonaerensis* co-occurs in the 'Serra da Mantiqueira' ecoregion (Minas Gerais state) but they are nonetheless easily distinguished in the field because the latter has paler (brownish grey, greyish orange to reddish grey) pileal squamules on a distinctly white pileus background, a more complex annulus with a fringed upperside, and smaller and mostly clavate to short clavate cheilocystidia (17−43 × 8−18 µm) (Spegazzini 1880, Singer 1951, Singer & Digilio 1951). *Macrolepiota colombiana*, in turn, resembles *M. pernuda* in the dark brown to blackish callote, caused by the surface-breaking around the center, but deviates from the latter in the smaller basidiomata (maximum reported pileus diameter of 120

mm, maximum stipe dimensions are 150 × 13 mm) with a banded brownish stipe, the grayish-red staining context on exposure, and the more complex annulus (often with doble edge) (Franco-Molano 1999). Interestingly, in a photograph from an authentic collection (Figure 15A) of *M. colombiana*, kindly provided by A.E. Franco Molano, a distinct volval patch could be identified on the surface of the still closed pileus of an unmature basidiomata. Although this trait was overlooked in the original description, it is also useful in distinguishing *M. colombiana* from *M. pernuda.* Microscopically, *M. colombiana* appears to have narrower basidiospores on average than *M. pernuda*, albeit with a higher inter-individual variability and Q_m value, and delivers a different stipe covering architecture, with terminal to subterminal elements predominantly tapering towards apex, whereas *M. colombiana* bears oblong to cylindrical terminal elements with an obtuse apex (Franco-Molano 1999). Furthermore, *M. colombiana* distribution seems to be restricted so far to northern South America (Franco-Molano 1999, Souza *et al*. 2022). Indeed, there are no reliable reports confirming the occurrence of *M. colombiana* outside Colombia. Judging by the descriptions and photographic representation, previous records of *M. colombiana* from Argentina, Brazil, and Chile (Ferreira e Cortez 2012, Putzke *et al*. 2014, Lining *et al*. 2021) clearly deviates from its authentic morphological concept (Figure 15), and in the absence of molecular analysis, they most likely refer to different species (viz., *M. abruptibulbosa*, *M. bonaerensis*, *M. capelariae*, *M. chapeleta,* or *M. pernuda*). A broader taxon sampling of *M. colombiana* is obviously necessary to precise its ecological/biogeographical range.

Macrolepiota abruptibulbosa C. Coelho-Nascimento & Menolli, **sp. nov._** (Figs. 16, 17)
MycoBank No: 000000

Diagnosis: *Macrolepiota abruptibulbosa* differs from *M. chapeleta* in the smaller basidiomata (maximum reported pileus diameter of 150 mm, maximum stipe dimension is 300 × 16 mm), white to yellowish white pileus background, typically evident among the appressed-squamulose covering, wider versiform cheilocystidia (19−43 × 11.0−19.0 μm), and smaller basidiospores (12.9−15.5 × 9.1−10.6 μm).

Etymology: *abruptus* (L.) = abrupt; *bulbous* (L.) = having the shape of or resembling a bulb, bloated; referring to the distinctly abrupt-bulbous stipe base, even in young basidiomata.

Holotype: Brazil, Rio Grande do Sul, Cambará do Sul, Parque Nacional de Aparados da Serra, 05 April 2023, *J.M. Timm* ***NCC278*** (FIFUNGI 1165), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Figure 15. **A**. Authentic collection of *M. colombiana* by A.E. Franco Molano. **B**−**L.** Putative records of *M. colombiana* from Colombia and Ecuador. Data from GBIF/iNaturalist. **A**−**C**, **E**−**I**, **K**−**L**. Colombia. **D**, **J**. Ecuador. Photographs: **A**. A.E. Franco-Molano, **B**. ©Fundación Dodo Colombia, **C**. ©Teodoro Chivatá Bedoya, **D**. ©Cecibel Cambisaca, **E**. ©Steven Cifuentes, **F**. ©William Gómez, **G**. ©Camilo Arturo, **H**. ©Mateo Hernandez Schmidt, **I**. ©Mayra Hernandez Salazar, **J**. ©rocio_varga, **K**. © Teodoro Chivatá Bedoya, **L**. ©P.A. Hill.

Basidiomata large-sized. **Pileus** 80−210 mm wide, fleshy ovoid to ovoid-conic when young, expanding conic-convex to convex or plano-convex, with deflexed margin and a conspicuous umbo; surface dry, smooth, pale brown (6B2) to pale yellowish brown (5B2) when young, then slightly rimose or disrupting into small fibrillose-squamulose patches on a paler background, sometimes leaving a central calotte; central calotte/disk smooth, dark brown (6E3, 6F3) to blackish; squamular patches small, irregularly shaped, pale brown (6B2) to pale orange brown (5B3), irregularly arranged, usually appressed; background smooth, white (1A1) to yellowish white (1A2); margin equal, with whitish woolly fibrils, appendiculate. Lamellae free, crowded, ventricose to subventricose, up to 13.5 mm broad, white (1A1) to yellowish white (1A2) or light yellow (3A2); lamellulae truncate, of several ranks, unevenly distributed; edge entire, concolor. **Stipe** 150−300 × 10−16 mm, cylindrical and slightly tapering upwards, protruding into pileus, with a conspicuous basal bulb (20−50 mm wide), commonly with a tomentose layer at base; surface beige (4A3) to pale brown, sometimes white (1A1) to yellowish white (1A2) in the upper third, with adnate zig-zag bands over the whole length on whitish (1A1) background but vaguer and less contrasting at the apex than in middle and lower parts. **Annulus** superior, descending, membranous, white (1A1) to yellowish white (1A2), movable when mature. **Context** white (1A1) and dull in pileus, unchanging, thick (up to 15 mm); stipe context concolor, with a central white cottony strand, unchanging. **Odor** weak, more or less nutty-fungoid. **Taste** fungoid, not very distinct. **Spore print** white.

Basidiospores [210/7/7] (12.0−)12.9−15.5(−16.0) × (8.5−)9.1−10.6(−11.0) µm [$\mathbf{L_m}$= 14.1 µm; $\mathbf{W_m}$ = 9.9 µm; Q = (1.31−)1.35−1.50(−1.55); $\mathbf{Q_m}$ = 1.42], ellipsoid, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, and metachromatic in Cresyl blue; apiculus variable from sublateral to subapical, rather small; contents monogutullate. **Basidia** (35−)39−42(−48) × 14.3−15.3(−16.5) µm, 4-spored (rarely 2-spored), clavate, pedunculate, hyaline, thin-walled. *Lamella edge* sterile, with crowded cheilocystidia. **Cheilocystidia** (16.0−)19.0−43(−46) × (9.0−)11.0−19.0(−20) µm, hyaline, colorless, thin-walled, variable in shape, commonly catenulate, ovoid, clavate to broadly clavate, broadly oblong, ventricose or irregularly shaped, some with secondary septa. **Pleurocystidia** absent. **Pileus covering** an intricate trichoderm composed of 6–10 cells chains, branching; intercalary elements (13.5−)17.0−41(−46) × (7.8−)8.7−22(−24) µm, globose, ellipsoid, oblong, clavate or cylindrical, slightly thick- to thick-walled, with brownish yellow intracellular pigment, encrusting elements not observed; terminal elements 12.0−25(−32) × (5.5−)6.5−9.3 µm, mostly cylindrical to slender clavate, also ovoid, globose or oblong, slightly thick- to thick-walled, with brownish yellow intracellular pigment, secondary septa sometimes present. **Stipe covering** as a trichodermial layer composed of cylindrical, narrowly clavate or

lanceolate elements, 17.0−91(−95) × (5.0−)7.0−16.8 µm, slightly thick-walled, usually with secondary septa. **Clamp connections** common at the base of basidia and in the stipe context hyphae

Additional specimens examined: Brazil, Rio Grande do Sul, São Francisco de Paula, Campos de Cima da Serra, 05 April 2023, *J.M. Timm* ***NCC186*** (FIFUNGI 1164), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 02 June 2024, *J.M. Timm* ***NCC295*** (FIFUNGI 1163), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* Parque Natural Municipal da Ronda, 15 May 2024, *H. Gründemann* ***iNat #215959597*** (FIFUNGI 1166), GenBank [ITS]: 00000000; *ibid.* 14 June 2024, *H. Gründemann* ***iNat #222645313*** (FIFUNGI 1167), GenBank [ITS]: 00000000; *ibid.* 27 April 2024, *H. Gründemann* ***iNat #211097848*** (FIFUNGI 1168), GenBank [ITS]: 00000000; *ibid.* 27 April 2024, *H. Gründemann* ***iNat #211096291*** (FIFUNGI 1169), GenBank [ITS]: 00000000.

Notes: *Macrolepiota abruptibulbosa* is the third species in the /capelariae clade and it is so far restricted to Southern Brazil. Indeed, it may represent a range-restricted species with apparent affinity for pine forests in somewhat high altitudes (900−1200 m a.s.l.). Although common in its type location, sampling efforts to find this species in different Southern and Southeastern Brazil areas have failed. In the field, *M. abruptibulbosa* may be confused with *M. bonaerensis* because of the somewhat similar pileus surface. The two species also display similar basidiospore size (11−16.2 × 7.3−9.3 µm in *M. bonaerensis*), albeit very divergent in other morphological traits. *Macrolepiota abruptibulbosa* produces more robust basidiomata, a less complex annulus with a smooth upperside, and a pileus covering composed of distinctly smaller elements (17−41 × 8.7−22 µm). Moreover, *M. abruptibulbosa* also differs from *M. bonaerensis* in the typical presence of zigzag bands or squamulose girdles on the stipe surface.

Phylogenetically, *M. abruptibulbosa* is sister to the *M. chapeleta* (Figure 2), from which it differs by 14 evolutionary events (11 SNPs + 3 indel). From a morphological point of view, *M. abruptibulbosa* can be separated from *M. chapeleta* by the absence of orange tones on pileus covering and the presence of an abrupt-bulbous stipe base; whilst microscopically they are different by the wider versiform cheilocystidia (19.0−43 × 11.0−19.0 µm), smaller basidiospores (12.9−15.5 × 9.1−10.6 µm), and the higher proportion of slender elements in the pileus covering of *M. chapeleta*.

Habit, habitat, and distribution: In groups, saprotrophic, and terrestrial in pine forest. Only found in the state of Rio Grande do Sul, Southern Brazil.

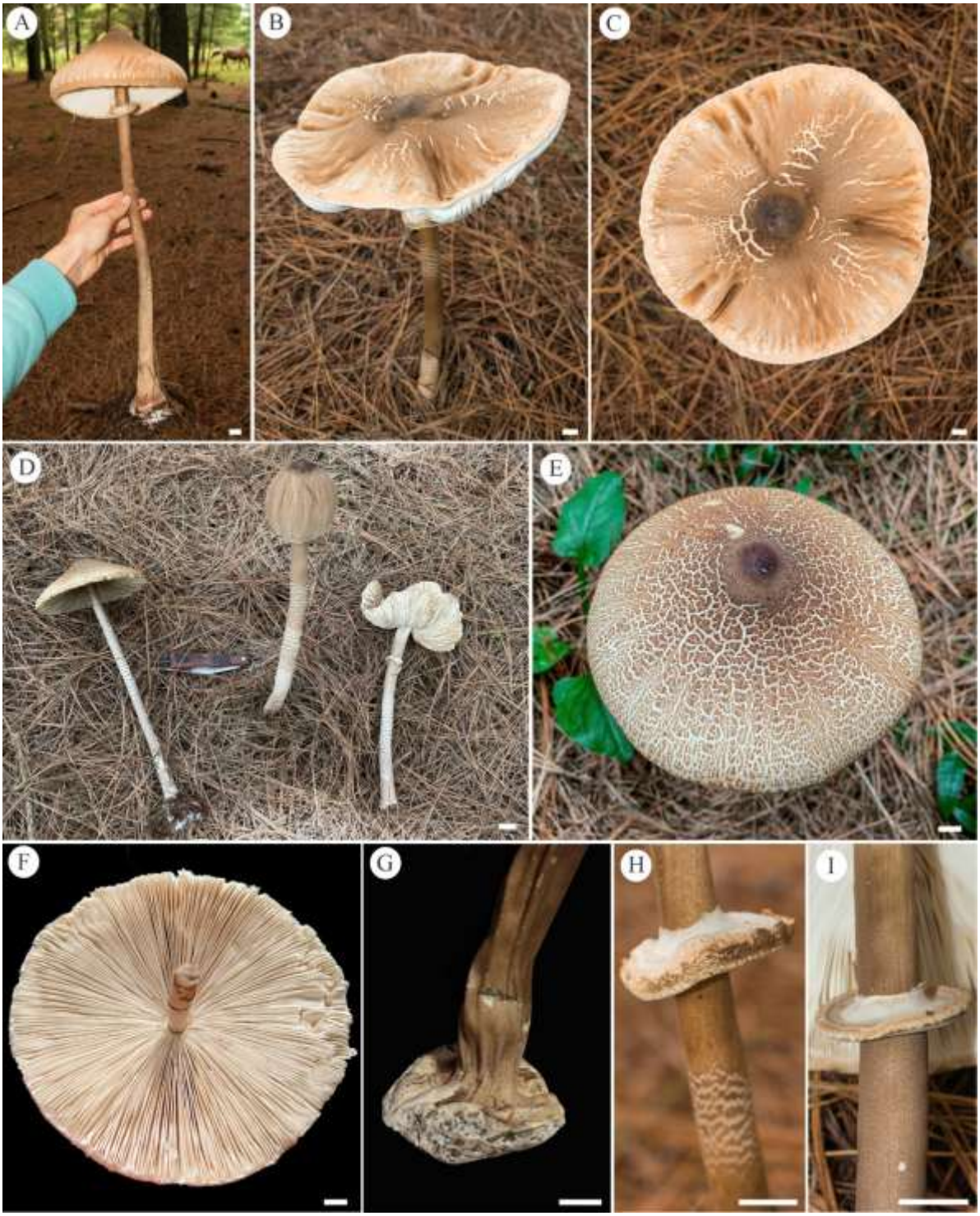

Figure 16. Basidiomata of *Macrolepiota abruptibulbosa* (NCC278, **FIFUNGI 1165**, holotype). Scale bars: A−B = 20 mm; C−I = 10 mm. Photographs: Jeferson M. Timm.

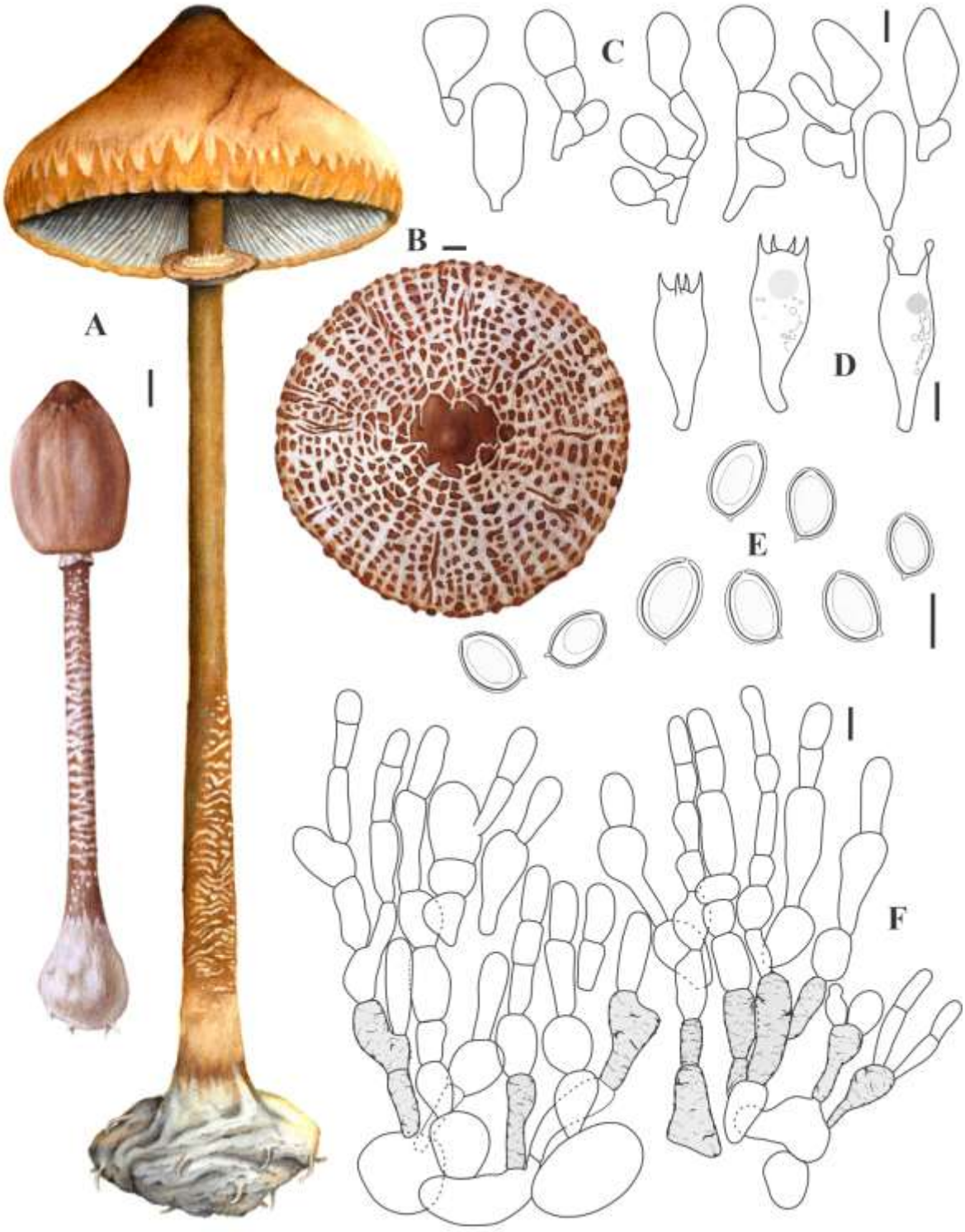

Figure 17. *Macrolepiota abruptibulbosa* (NCC278, **FIFUNGI 1165**, holotype). **A**. Basidiomata. **B**. Mature pileus in top view. **C**. Cheilocystidia. **D**. Basidia. **E**. Basidiospores. **F**. Pileus covering elements. Scale bars: **A**−**B** = 10 mm; **C**−**F** = 10 µm. Watercolor illustrations: Dyego Costa. Line drawings: Cristiano Coelho-Nascimento.

Macrolepiota pulchella* var. *gymnopodia C. Coelho-Nascimento & Menolli, **var. nov.**
(Figs. 18, 19)
MycoBank No: 000000

Diagnosis: *Macrolepiota pulchella* var. *gymnopodia* differs from the autonomous variety *Macrolepiota pulchella* var. *pulchella* by the presence of an abrupt basal bulb, the ellipsoid to cylindrical basidiospores, and the absence of a volva on the stipe base.

Etymology: *gymno* (Gk.) = naked; *podós* (Gk.) = foot; referring to the absence of a distinct volva or any volval remnants on stipe base.

Holotype: Brazil, São Paulo city, Parque Estadual da Cantareira, Núcleo Pedra Grande, 12 January 2022, *C. Coelho-Nascimento, D.A. Zabin & A.C. Morais* ***NCC178*** (FIFUNGI 1135), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Basidiomata medium-sized. **Pileus** 40−60 mm wide, freshy, convex to obtusely conic with age, umbonate, with low umbo, straight margin; surface dry, pale brownish grey ($N_{40}Y_{20}M_{10}$) at first, later covering breaking into a greyish brown ($N_{80}Y_{50\text{-}60}M_{20}$), dark greyish brown ($N_{90}Y_{10\text{-}20}M_{10\text{-}20}$), dark brown ($N_{90}Y_{40}M_{20,}$ $N_{99}Y_{50}M_{40}$) to blackish calotte at the center; calotte round to irregularly star-shaped, smooth to felted-subsquamulose, densely surrounded by small squamules with curled edges on a paler background; background pale brownish grey ($N_{40}Y_{20}M_{10}$) to pale greyish brown ($N_{30}Y_{30}M_{10}$), radially appressed-fibrillose; a white velar patch sometimes present. **Lamellae** free, remote, crowded, white to yellowish white ($N_{00}Y_{10}M_{00}$); lamellulae plentiful, with several ranks, truncate, unevenly distributed. **Stipe** 70–120 × 4–5 mm, central, cylindrical to slightly widening downward, bulbous at base; bulb 12–15 mm wide, oblong to flattened submarginate, with short white rhizomorphs; surface glabrous, longitudinally fibrous striate, white to yellowish white ($N_{00}Y_{10}M_{00}$) at base, elsewhere pale brown ($Y_{40}M_{30}C_{30}$) to light greyish brown ($Y_{40}M4_0C_{40}$), with whitish spots with age. **Annulus** apical**,** flaring upwards and away from the stipe (ascending), membranous, whitish upperside with a brownish and squamulose edge. **Volva** absent. **Context** 3.5–4.0 mm thick and white in pileus, unchanging; white and hollow in stipe, unchanging. **Odor** mild, fungoid. **Taste** not recorded. **Spore print** white.

Basidiospores [90/3/3] 9.1−11.9(−12.2) × 5.1−6.8(−7.2) µm [$\mathbf{L_m}$= 10.4 µm; $\mathbf{W_m}$ = 6.2 µm; Q = (1.42−)1.44−2.04(−2.10); $\mathbf{Q_m}$ = 1.68], ellipsoid to cylindrical, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous,

metachromatic in Cresyl blue; apiculus sublateral to subapical, apex subobtuse or truncate at the apex, contents granular or multi-guttulate. **Basidia** 25−38 × 10−15.5 µm, clavate to broadly clavate, hyaline, thin-walled, 4-spored. **Lamella edge** composed of a broad sterile zone of very crowded cheilocystidia. **Cheilocystidia** 20−59 × 4.5−10 µm, mostly narrowly fusiform, fusiform to lanceolate, less frequently subclavate, narrowly cylindrical or narrowly utriform, often catenate, hyaline, colorless, thin-walled. **Pleurocystidia** absent. **Pileus covering** an intricate trichoderm composed of erect to suberect elements; intercalary elements highly branching, thin- to slightly thick-walled, with a pale brownish yellow intercellular pigment, mostly cylindrical, narrowly cylindrical to narrowly fusiform (20−) 25–63.3 × 4.1–6.5 µm, sometimes narrowly clavate or oblong 17−40 × 8−14.8 µm, rarely irregular shaped; terminal elements mostly as clusters of 4−5 narrowly cylindrical to narrowly fusiform cells resembling the fingers of a glove; basal elements mostly as clavate, broadly clavate to subglobose elements 20−44 × 15.8−28 µm. **Stipe covering** composed of mostly repent elongated cylindrical hyphae, 2–10 µm in diam, hyaline, thin-walled, with a pale brown vacuolar pigment. **Clamp connections** not observed in all tissues.

Additional specimens examined: Brazil, Minas Gerais, Camanducaia, Serra da Mantiqueira, Sítio Mandaçaia, 23 October 2023, *C. Coelho-Nascimento & T.V.D.H. Comenale* ***NCC287*** (FIFUNGI 1137), GenBank [ITS]: 00000000; São Paulo, Campos do Jordão, Parque Estadual Campus do Jordão (PECJ), Trilha do Rio Sapucaí, 21 January 2020, *M.P. Drewinski*, *N. Menolli Jr.*, *M.F.O. Silva*, *M.P.C. Santos* & *T.R. Santos* ***NMJ284*** (FIFUNGI 1136), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Specimens examined of *Macrolepiota pulchella* var. *pulchella*: Brazil, São Paulo, São Paulo city, Parque Estadual das Fontes do Ipiranga, 9 October 2001, *U.C. Peixoto* ***PEFI13/2001*** (SP381604); *ibid.* 21 September 2005*, C. Puccinelli & U.C. Peixoto* ***CP 161*** (SP466948); *ibid.* 3 October 2006, *C. Puccinelli* ***CP193*** (SP381606); 13 August 2006, *L.J. Gimenes* ***LJG130/06*** (SP381605); *ibid.* 13 November 2007, *N. Menolli & F. Karstedt* ***NMJ155*** (SP466946); *ibid.* 19 October 2011, *J.J.S. Oliveira & P.O. Ventura* ***JJSO386*** (SP466949), GenBank [ITS]: MG136891; São Paulo city, Parque Estadual da Cantareira, 23 October 2008, *M. Capelari & L.A.S. Ramos* ***MC4411*** (SP466947), GenBank [ITS]: MG136894; *ibid.* Itapecerica da Serra, 10 July 1997, *A.M. Gugliotta* ***AMG945*** (SP466950); *ibid.* 24 July 1997, *M. Capelari & A.M. Gugliotta* ***MC3955*** (SP466951). Santa Catarina, Florianópolis, Morro da Lagoa, 13 April 2012, *S. Urrea-Valencia* ***124*** (FLOR 50954); *ibid.* 16 October 2012, *S. Urrea-Valencia* ***176*** (FLOR 51003).

Figure 18. Basidiomata of *Macrolepiota pulchella* var. *gymnopodia*. **A**–**F**, **H**. NCC178 (**FIFUNGI 1135**, holotype). **G**. NMJ284. Scale bars: 10 mm. Photographs: **A**–**F**, **H**. Cristiano Coelho-Nascimento. **G**. Nelson Menolli Jr.

Habit, habitat, and distribution: saprotrophic, terrestrial, and growing solitary or gregarious in fragments of Dense or Mixed Ombrophilous Forest. *Macrolepiota pulchella* var. *gymnopodia* is currently known from the states of Minas Gerais and São Paulo (Southeastern Brazil), where it co-occurs with its typical variety that was originally described from the state of Rio Grande do Sul (Southern Brazil).

Notes: In the protologue, *M. pulchella* was described as *Lepiotella brunnea* Rick, from the state of Rio Grande do Sul, Southern Brazil, as having white lamellae, a persistent annulus, and a volvate stipe base (Rick 1938). The short diagnosis given by Rick do not contain any detail about microscopic characters, and, thus, it does not directly evoke the generic concept of *Macrolepiota*. In Singer (1953) type studies, an authentic material (no. 20592) determined as *L. brunnea* by Rick was analyzed. Macroscopically, Singer (1953) described *L. brunnea* in having a squarrulose pileus surface, an adnate annulus, and a distinct saccate and membranous volva; whilst, microscopically, only the common presence of clamp connections and ellipsoid basidiospores (10.5 × 7.5 µm) with a germ-pore are reported. Singer (1953) retained provisionally Rick's name but he suspected that *Lepiotella* could be a synonym of *Macrolepiota* or *Clarkeinda* Kuntze. Later, Singer (1959) replaced *Lepiotella* by *Volvolepiota* Singer, which was further synonymized with *Macrolepiota* by Vellinga & Yang (2003).

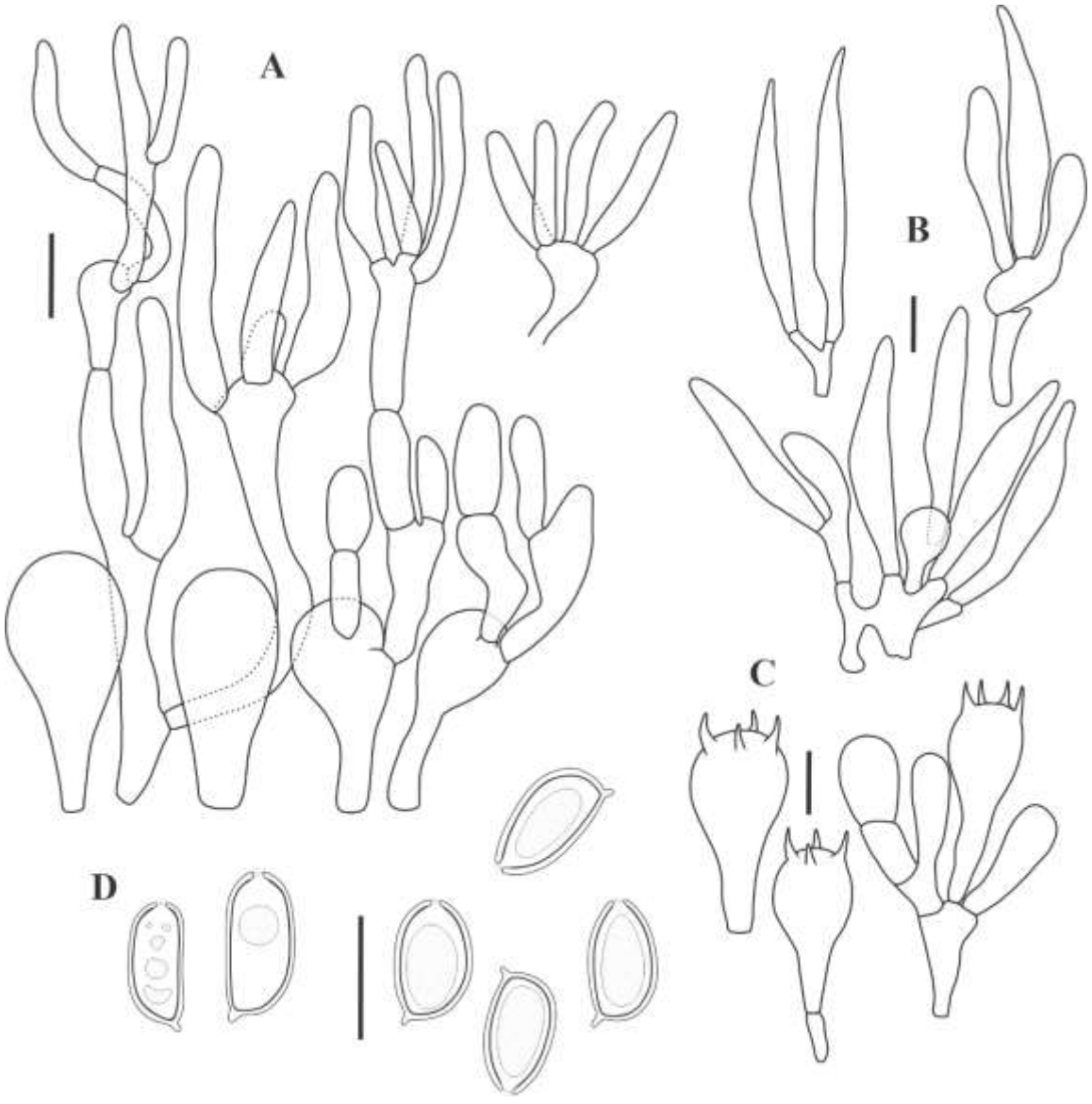

Figure 19. *Macrolepiota pulchella* var. *gymnopodia* (NCC178, **FIFUNGI 1135**, holotype). **A**. Pileus covering elements. **B**. Cheilocystidia. **C**. Basidia. **D**. Basidiospores. Scale bars = 10 μm. Line drawings: Cristiano Coelho-Nascimento.

The first complete morphological description of *M. pulchella* was provided by Heinemann & de Meijer (1996) based on a unique basidioma (de Meijer 2935) from the state of Paraná, Southern Brazil. Freitas & Menolli (2019) provided an additional complete description based on several collections (herein re-examined) from the state of São Paulo, Southeastern Brazil. These authors reported the first occurrence of *M. pulchella* outside Southern Brazil and yields the first molecular data to the species. Judging from the aforementioned descriptions and their plates, the presence of a distinct membranous volva and mostly ellipsoid basidiospores clearly represent consistent characters among the sampled specimens, although some inconspicuously volvate basidiomata were referred by Heinemann & de Meijer (1996). Interestingly, three recent collections found in the states of São Paulo and Minas Gerais (NCC178, NCC287, NMJ284) were completely devoid of any volval remnants (Figure 18). Additionally, these collections exhibited a distinct abrupt bulbous stipe base and a prevalence of elongate to cylindrical basidiospores [Q = (1.42−)1.44−2.04(−2.10); $\mathbf{Q_m}$ = 1.68]. Here we decided to attaches some taxonomical importance to these traits and thus propose a new variety named *M. pulchella* var. *gymnopodia*.

Macrolepiota sabulosa Fazolino & R.M. Silveira, Mycologia 110 (5): 930. (Fig. 20)

= *Macrolepiota sabulosa* var. *velistellaris* D.S. Freitas & Menolli, Mycotaxon 134(2): 234 (2019).

Holotype: Brazil, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, Trilha da Geologia, Sítio Mandaçaia, 06 June 2016, *E. Fazolino Perez **000689*** (UFRN-FUNGOS 2693!).

Basidiomata large-sized. **Pileus** 130−185 mm wide, freshy, ovoid to ovately conic when young, expanding to convex or plano-convex, with a low umbo at disc; surface dry, brownish grey (7D2, 7DE2) to greyish brown (7E3) at first, darker at center, later covering breaking into a central calotte and either small appressed-squamulose patches or radially arranged appressed-fibrillose squamules on a paler background; calotte round, smooth, dark brown (6F4, 6F5), dark greyish brown (6F3) to blackish, sometimes partially covered by a membranous whitish velar patch; covering elsewhere brownish grey (7E2) to greyish brown

(7E3); background dirty white or beige (4A3) to brownish gray (5C3), smooth to minutely appressed-fibrillose. **Lamellae** free, remote, crowded, subventricose, up to 10 mm broad, yellowish white (1A2) to cream (4A3) and plentiful bluish black stains with age; lamellulae rare or absent; edge entire and concolor. **Stipe** 100−170 × 8−12 mm, slightly widening downwards to an abrupt, slightly flattened subglobose bulb**,** up to 26 mm wide, stuffed then hollow; surface densely covered up to the apex with dark brown (7F4-5) appressed squamules on a pale beige (4A2) background. **Annulus** superior to apical, membranous, sometimes with a broad white cuff-like part around stipe, whitish upperside; underside pale brownish grey (9C2) with a dark brown (6F4) squamulose edge. **Volva** limbate, membranous with a cottony base, dirty white. **Context** up 5.0 mm thick and white in pileus, unchanging; grayish white (1B2) in stipe, unchanging. **Odor** and **taste** not recorded. **Spore print** white.

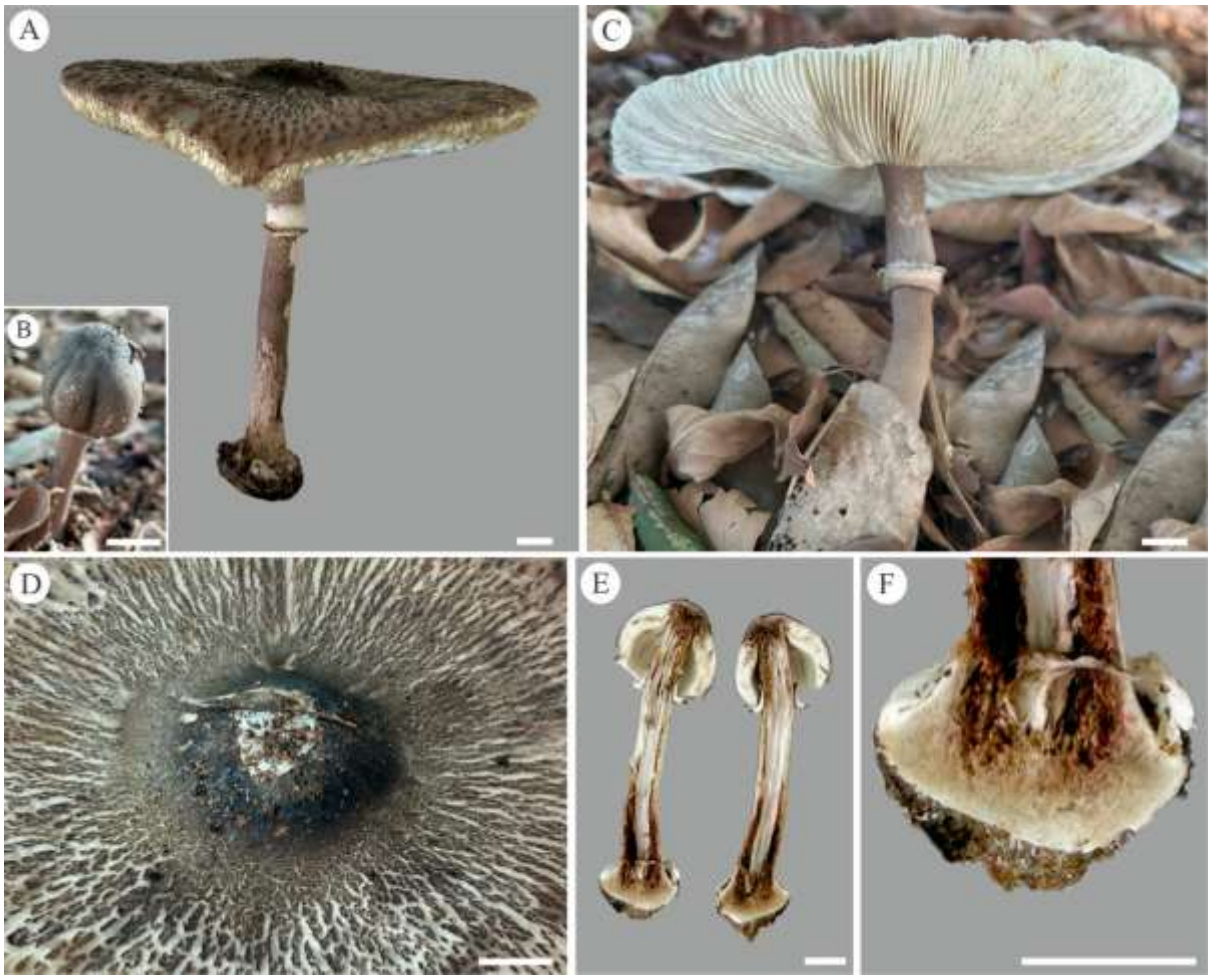

Figure 20. Basidiomata of *M. sabulosa*. (NCC282). **A**, **C**. Mature basidioma. **B**. Young basidioma. **D**. Volval patch at disc. **E**. Young basidiome in longitudinal section. **F**. Bulb and volva detail. Scale bars: 10 mm. Photographs: Miguel D. Xavier.

Basidiospores [150/5/5] (7.8−)8.9−12.5(−14) × (5.0−)5.2−6.5(−8.0) µm [**L_m**= 9.5 µm; **W_m** = 5.6 µm; Q = (1.44−)1.58−1.92(−1.98); **Q_m** = 1.70], ellipsoid to elongate, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, metachromatic in Cresyl blue; apiculus subapical, contents monogutullate to multigutullate. **Basidia** 24−39 × 9−15 µm, clavate, hyaline, thin-walled, 4-spored. **Lamella edge** composed of a broad sterile zone of very crowded cheilocystidia. **Cheilocystidia** (25−)29−56(−60) × (6.5−)6.9−10.5 µm, slender clavate to cylindrical, infrequently rostrate, thin-walled, rarely with secondary septa. **Pleurocystidia** absent. **Pileus covering** a trichoderm composed of erect, densely packed hyphae 150–185 µm long, septate, with thin to slightly thickened walls, with a diffuse pale greyish brown to yellowish brown pigment; terminal elements predominantly cylindrical with round apex, but also slender clavate, subfusiform or tapering towards apex 30–58.5 × 6.9–11.0 µm; intercalary and basal elements often inflated, thin-walled or slightly thick-walled, predominantly oblong, cylindrical to clavate, but also globose, subglobose or broadly oblong, 19–65 × 7.0–25 µm. **Stipe covering** composed of bundles of multiseptated hyphae, with a diffuse pale brownish pigmentation, with thin to slightly thickened walls; terminal elements predominantly cylindrical with round apex, less frequently tapering towards apex, 25–70 × 5–9 µm. **Clamp connections** observed at the base of basidia, basidiole, and cheilocystidia, also at the hyphae of the stipe context.

Line drawings: see Fazolino Perez *et al*. (2018) and Freitas and Menolli (2019).

Additional specimens examined: Brazil, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, Trilha Ubaia Doce, 20 January 2016, *M.D. Xavier **70*** **(**UFRN-FUNGOS 2694, paratype); *ibid.* Trilha da Geologia, 12 January 2008 *J.J.S. Oliveira s.n.* (UFRN-FUNGOS 935); Natal, Mata da Caern, UFRN - Campus Universitário Central, 22 July 2023, *C. Coelho-Nascimento & P.G.R Xavier **NCC282*** (FIFUNGI 1156), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 23 July 2023, *C. Coelho-Nascimento & P.G.R Xavier **NCC283*** (FIFUNGI 1157), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; Baía Formosa, Mata Estrela, Reserva Particular de Patrimônio Natural Mata Estrela, 14 July 2010, *N. Menolli. & al. **NMJ185*** (SP466945 type of *Macrolepiota sabulosa* var. *velistellaris*).

Habit, habitat, and distribution: saprotrophic, terrestrial, growing solitary to small group in sandy soil among dry foliage. In Dense Ombrophilous Forest or Lowland Semideciduous

Seasonal Forest within the Atlantic Rainforest domain. Only known from the state of Rio Grande do Norte, Northeastern Brazil.

Notes: This phylogenetically well delimited species (Figure 2) has been formerly identified described as *M. sabulosa* from the state of Rio Grande do Norte, Northeastern Brazil, and recognized as a distinct lineage by Fazolino Perez *et al*. (2018), although in its original description the authors did not refer the presence of a volva at the stipe base. Subsequently, Freitas and Menolli (2019) proposed a new variety of *M. sabulosa* based on the distinct presence of a volva from a collection (SP466945) also found in the state of Rio Grande do Norte. Likewise, the examination of recent collections (NCC282 and NCC283) of *M. sabulosa* from the type locality found the presence of a basal membranous volva, which was also observed in our re-examination of the type collections (UFRN 2693 and UFRN 2694). Taken together, these finds indicate that the presence of a volva was overlooked in the *M. sabulosa* original description by Fazolino Perez *et al*. (2018). Therefore, we here interpret *M. sabulosa* var. *velistellaris* as a synonym of the autonomous variety *M. sabulosa* var. *sabulosa*, which can be interpreted as a volvate species.

A complete taxonomic description of *M. sabulosa* is provided above, with a particular emphasis on providing additional information on basidiospores, cheilocystidia, and pileus covering elements. The latter character architecture, for example, is not limited to cylindrical uninflated hyphae (Fazolino Perez *et al*. 2018) but also including globose, broadly oblong, and clavate elements. Additionally, a conspicuous and diffuse brownish pigment was found throughout the trichodermal layer but no spirally-incrusting pigment was observed.

Macrolepiota cyanolamellata Fazolino, Lechner & Suaza Blandón, Mycologia 110 (5): 930. (Fig. 21)

Holotype: Brazil, Rio Grande do Sul, Porto Alegre, Morro Santana, 05 May 2015, *E. Fazolino Perez* ***000516*** (ICN 187662!).

Basidiomata medium to large-sized. **Pileus** 75−110 mm wide, freshy, ovoid to subglobose or hemispherical when young**,** plano-convex to slightly uplifted when mature with low umbo; surface dry, dark brown (7F4-5) to reddish brown (8F4-5) at first, later covering breaking into a dark greyish brown (8F3) to blackish calotte at the center; callote round, rugulose to felted-squamulose; around central callote with dense small squamules (paler than the callote), adnate or with recurving edges on all sides, on a fibrillose to coarsely fibrillose,

whitish background; margin slightly to distinctly fringed and exceeding lamellae. **Lamellae** free, remote crowded, white (1A1) to grayish white (1B1) or yellowish white (1A2), exhibiting bluish tones (21B1, 21B2) with age, mainly at the edges; lamellulae rare or absent. **Stipe** 100–150 × 0.6–2.0 cm, cylindrical, central, slightly widening downwards to a clavate to bulbous base 16–30 mm wide, stuffed then hollow; surface densely covered up to the apex with dark brown (7F4-5) or reddish brown (8E3-4) appressed squamules on a whitish to pale beige (4A2) background. **Annulus** superior, ascending, complex, thickened, with a cuff around stipe; upperside white (1A1) and fringed; underside smooth, white (1A1), dirty white to pale brownish grey (6C2), with a brownish squamulose edge. **Volva** limbate, membranous with a cottony base, dirty white. **Context** up 3.5 mm thick and white in pileus, unchanging; grayish white (1B1) in stipe, unchanging. **Odor** mild, fungoid. **Taste** not recorded. **Spore print** white.

Figure 21. A−**F** Basidiomata of *M. cyanolamellata*. **A**−**C**. NCC292. **D**−**F**. MC518. Scale bars: 10 mm. Photographs: **A**–**C**. Larissa Trierveiler-Pereira, **D**–**F**. Michelle G. Campi.

Basidiospores [150/8/4] (9.5−)10−15.3(−21) × (6.0−)6.5−10(−13) µm [$\mathbf{L_m}$= 12 µm; $\mathbf{W_m}$ = 7.7 µm; Q = (1.38−)1.43−1.73(−2.27); $\mathbf{Q_m}$ = 1.58], ellipsoid to oblong, some elongated

and tapered, thick-walled, with a germ pore covered by a hyaline cap, smooth, hyaline, thick-walled, weakly dextrinoid, congophilous, metachromatic in Cresyl blue; apiculus subapical, contents monogutullate to multigutullate. **Basidia** 28–39 × 9.5–13 µm, clavate, hyaline to pale brownish orange, thin-walled, 4-spored. **Lamella edge** composed of a broad sterile zone of very crowded cheilocystidia. **Cheilocystidia** 22–48 × 6.5–12 µm, in bundles, predominantly clavate to narrowly clavate, less frequently fusiform, sometimes 1-septate, hyaline or with a golden-brown pigment in KOH, in clusters. **Pleurocystidia** not observed. **Pileus covering** a trichoderm composed of erect to suberect hyphae 180–303 µm long, septate, thin- to slightly thick-walled, with a diffuse pale brownish to pale reddish pigment; terminal elements predominantly cylindrical with round apex or slender clavate, less frequently tapering towards apex, 12.5–78 × 6.8–11.5 µm; intercalary and basal elements often inflated, mostly cylindrical to cylindrical-sinuous, rarely irregular shaped, 10.5–58 ×5.7–11.9 µm. **Stipe covering** elements similar to those on the pileus, terminal elements 29.5–71.0 × 6.5–10.5 µm. **Clamp connections** rare to frequent at the hyphae of the stipe context and in base of basidia and cheilocystidia.

Line drawings: see Fazolino Perez *et al*. (2018).

Additional specimens examined: Brazil, São Paulo, Angatuba, Bairro Guareí Velho, Mata do Zé Branco, 15 December 2023, *Trierveiler-Pereira, Baltazar & C. Coelho-Nascimento* ***NCC292*** (FIFUNGI 1162), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000. Rio Grande do Sul, Porto Alegre, Morro Santana, 2 May 2015, *A.C. Magnago* ***1165*** (ICN 187663, paratype). Paraguay, Central Department, Aregua, 9 June 2020, *M. Campi* ***MC518*** (FACEN5644), GenBank [ITS]: 00000000, [nLSU]: 00000000, [*tef1-α*]: 00000000.

Habit, habitat, and distribution: Terrestrial, saprotrophic, solitary, and growing on soil. In Semideciduous Seasonal Forest, within the Atlantic Rainforest domain in Brazil, and in a Humid Chaco area in Paraguay. Known from Paraguay Central Department and the Brazilian states of Rio Grande do Sul e São Paulo.

Notes: As phylogenetically circumscribed here, this species constitutes a well-supported clade (MLB = 86) sister to *M. sabulosa* within the *Macrolepiota* sect. *Velosa* (Figura 2). Interestingly, two recent collections of *M. cyanolamellata* from Brazil and Paraguay (NCC292 and MC518, respectively) exhibited the presence of a distinct volva at the stipe base, which was also observed in our type collections study (ICN 187662, ICN 187663) (Figure 22). These findings

confirm that the presence of a volva was overlooked in the original description of *M. cyanolamellata* (Fazolino Perez *et al*. 2018).

Figure 22. *Macrolepiota cyanolamellata* type collection at the ICN herbarium (ICN 187663) showing the presence of a distinct volva at the stipe base. Photograph: ICN herbarium archive.

Dichotomous key for the currently known species of Macrolepiota *from the Neotropics*

1. Stipe never more than twice pileus diameter; volva present at base of stipe.....**2**
1'. Stipe usually more than twice pileus diameter; volva absent from the base of stipe.....**5**

2. Basidiomata large-sized (mature pileus × stipe ≥ 70 diam. × 135 mm long); lamellae with bluish tinges when aged (mainly at the edge); stipe covering medium to dark brown, fibrillose-squamulose, without zigzag bands or squamulose girdles.....**3**
2. Basidiomata consistently medium-sized (mature pileus × stipe ≤ 70 diam. × 135 mm long); lamellae without bluish tinges; stipe covering pale brown to light greyish brown, longitudinally fibrous striate, usually with inconspicuous adnate zigzag bands.....**4**

3. Annulus ascending, thick, complex, upperside whitish and fringed, underside pale tan with a dark brown edge; basidiospores measuring (7.5–)9.0–17.0(–19.5) × (4.5–)6.0–9.0(–11.0) µm; restricted to Northeastern Brazil on sandy soil habitats.....***M. cyanolamellata***

3'. Annulus descending, thin, simple, whitish with a brownish underside edge; basidiospores measuring (8.5–)9.5–13.0(–15.0) × (5.0–)5.5–7.5(–11.5) µm.....***M. sabulosa***

4. Pileus covering with greyish brown to blackish granular squamules lying on top of a radially fibrillose background; cheilocystidia mostly narrowly fusiform to fusiform or lanceolate .....***M. pulchella***
4'. Pileus covering with appressed brownish squamules dissociating radially on top of a fibrillose background; cheilocystidia cylindrical to narrowly clavate.....***M. dunensis***

5. Annulus thickened, often with a double edge (crown), whitish and fringed to fibrillose upperside; pileus surface predominantly formed by a white and strongly radially fibrillose background.....**6**
5'. Annulus usually thin and simple, less frequently slightly thickened with a double edge, whitish and smooth upperside; pileus surface background smooth or slightly fibrillose, mostly covered by a squamulose and/or patchy covering.....**7**

6. Stipe covering brown to dark brown, smooth to rugulose, with adnate zigzag bands or squamulose girdles usually over the entire length and contrasting in color with the background; stipe context white, slowly becoming greyish-red when exposed.....***M. colombiana***
6'. Stipe covering whitish orange to brownish grey, paler at the apex, farinose-furfuraceous to locally rimulose, rarely with adnate zig-zag bands; stipe context unchanging.....***M. bonaerensis***

7. Basidiospores < 15 ×11 µm, Q_m < 1.5; pileus covering usually with crust-like or uplifted squamulose patches.....**8**
7'. Basidiospores often > 15 ×11 µm, Q_m > 1.5; pileus covering barely breaking or breaking up into radially interwoven strips, rarely forming squamulose patches.....**9**

8. Pileus surface background glabrous; stipe with an abrupt basal bulb; cheilocystidia variable in shape from ovoid, clavate to broadly clavate, broadly oblong, ventricose or irregular shaped.....***M. abruptibulbosa***
8'. Pileus surface background appressed-fibrillose; stipe base only slightly enlarged; cheilocystidia mostly clavate to slender clavate or short clavate, sometimes cylindrico-clavate.....***M. pernuda***

9. Pileus covering light brown to pale greyish brown, brownish orange or clay brown, breaking up into radially arranged interwoven strips, microscopically as a trichoderm of mostly clavate to narrowly clavate terminal elements; basidiospores measuring (8.8−)12.5−20(−21) × (5.8−)7.5−11.7(−12.0) µm.....***M. capelariae***

9'. Pileus covering light orange to golden yellow or grayish orange, barely breaking up, microscopically as a trichoderm of mostly globose, subglobose to ellipsoid elements; basidiospores measuring (19−)20−26(−30) × (11.5−)12.1−14.5(−15.2) µm.....***M. chapeleta***

Ethnomycology

Demographic characteristics of informants

Among 22 informants who participated in the survey, 14 are men (63.6%) and eight are women (36.4%). The sample's mean age was 51 years old (y/o), with the average age of men 55 y/o and the average age of women 46 y/o, whilst the maximum age was 78 y/o and the minimum was 28 y/o. The respondents' age category with the greatest representation was in the 41−60 age bracket (11,5%). At the time of the survey, most informants were married (72.7%). Regarding educational attainment, all respondents were literate, with 18.8% having completed only the primary school level and 81.2% having achieved relatively high levels of education. The ethnic composition of the respondents included Germans (8), Poles (5), and Dutch (2) Brazilians, in addition to seven informants who did not self-identify as belonging to any ethnic group. Most informants were farmers (9), while some were merchants (4), civil servants (3), homemakers (4), and a few were laborers (2).

Diversity, recognition, and folk taxonomy of wild edible species of Macrolepiota

The wild edible species of *Macrolepiota* are a common food source for all survey respondents from Southeastern and Southern Brazil, with most of them residing in the Parelheiros district, São Paulo city. In total, 22 collections of wild edible *Macrolepiota* were obtained from guided expeditions conducted in the aforementioned area and other localities where key informants were resident. Following preservation, the collections were subjected to morphological and molecular analyses, resulting in identification of six distinct species (Table 2). Among these species, three represent species new to science that were formally introduced above in the 'Taxonomy' section (viz. *M. abruptibulbosa*, *M. chapeleta*, and *M. pernuda*). The remaining species are represented by previously known taxa, namely *M. bonaerensis*, *M. capelariae*, and *M. pulchella*. Of these, the latter two are documented for the first time as edible

resources, whereas *M. bonaerensis* have been regarded previously as edible (Singer 1951, Singer & Digilio 1951). *Macrolepiota bonaerensis* was the most frequently harvested during the forays, and it was referred to as the "giant white mushroom" of the grazing lands.

Table 2. Wild edible *Macrolepiota* species well recognized in the informants' areas, and their habitat and uses.

Scientific name	Collector number (Voucher at fungarium)	Location (State*)	Habitat	Uses
M. bonaerensis	**NCC183 (**FIFUNGI 1144**)**; **NCC269 (**FIFUNGI 1141**)**; **NCC270 (**FIFUNGI 1143**)**; **NCC288 (**FIFUNGI 1138**)**; **NCC289 (**FIFUNGI 1139**)**; **NCC290 (**FIFUNGI 1140**)**	MG, SP, RS	Grazed pastures, and other grassland habitats	Culinary, income
M. capelariae	**NCC184 (**FIFUNGI 1145**)**; **NCC185 (**FIFUNGI 1146**)**; **NCC285 (**FIFUNGI 1148**)**; **NCC291 (**FIFUNGI 1153**)**	MG, SP, RS	DOF**, MOF***	Culinary, income
M. abruptibulbosa	**NCC186 (**FIFUNGI 1164**)**; **NCC278 (**FIFUNGI 1165**)**; **iNat 215959597 (**FIFUNGI 1166**)**; **iNat 222645313 (**FIFUNGI 1167**)**; **iNat 211097848 (**FIFUNGI 1168**)**; **iNat 211096291 (**FIFUNGI 1169**)**	RS	MOF (in pine dominated areas)	Culinary
M. chapeleta	**NCC187 (**FIFUNGI 1130**)**; **NCC294 (**FIFUNGI 1132**)**	SP	DOF	Culinary, income
M. pernuda	**NCC201 (**FIFUNGI 1159**)**; **NCC205 (**FIFUNGI 1158**)**; **CT03 (**FIFUNGI 1161**)**	MG	MOF	Culinary
M. pulchella	**NCC287 (**FIFUNGI 1137**)**	MG	DOF	Culinary

** Brazilian states: MG = Minas Gerais, RS = Rio Grande do Sul, SP = São Paulo;*
*** **DOF** = Dense Ombrophilous Forest; ***__MOF__ = Mixed Ombrophilous Forest.*

All respondents demonstrated an adequate comprehension of how to distinguish the *Macrolepiota* species from other mushroom genera that are present in their community. Reported diagnostic features of *Macrolepiota* species include: (1) terrestrial habit; (2) distinctly long-stalk mushrooms with a stout apical cap; (3) squamulose cap surface; (4) white, unchanging gills; and (5) stipe surface with zigzag horizontal bands. They were also able to recognize the different sites where the species grow, including their phenology.

Regarding species identification and folk taxonomy, most informants (64%) recognize the presence of at least two distinct local "varieties" (ethnotaxa) based on their differential occurrence in grassland habitats or forest fragments, as well as divergent color and stature; meanwhile 36% of respondents maintain that they usually harvest the same single species, as they consistently spot the mushrooms in the same area (or in a nearby location) and during the same season. Despite the simple folk classification, some interesting vernacular names were found to be commonly ascribed to wild edible *Macrolepiota* species, such as "chapéu-de-sol"

(sunhat), “guarda-sol” (parasol), “chapéu-de-cobra” (snake hat), “chapeleta” (a small hat with a narrow brim), “pernuda” (who has long legs), and “cogumelo-frade” (friar mushroom). Scientific names were also employed by some respondents to identify harvested specimens, including *M. colombiana*, *M. procera*, and *M. mastoidea*. The latter two names were mentioned by 54.5% and 40.1% of respondents, respectively. These respondents were of German or Polish descent, while the name *M. colombiana* was mentioned only by non-ethnic identified respondents (36,4%).

The use and preparation methods of wild edible Macrolepiota *mushrooms*

The only recognized use of wild *Macrolepiota* mushrooms is for food (Figures 23−25). Among the respondents, the following cooking methods were reported for preparing these wild edible mushrooms: (1) sautéing the mushroom caps (either whole or sliced) in butter or olive oil and seasoning them with salt and black pepper and/or other inexpensive spices, such as garlic, onion, curry, nutmeg, and saffron; (2) grilling or broiling the mushroom caps, with only salt added as seasoning; (3) braising or stewing sliced mushroom caps with precooked meat, fish or poultry; and (4) pan-frying the mushroom caps in butter or olive oil, sometimes sealing them in a mixture of wheat flour, beaten egg, and breadcrumbs before frying. It is also worth mentioning that a single informant attested to the utilization of *M. bonaerensis* in the preparation of a homemade version of a garum (traditional Roman fish sauce). The recipe for this garum involves blending fresh mushrooms, koji, and salt, which allows the mixture to ferment for six weeks before straining the liquid. The resulting mushroom-based sauce was reported to enhance umami in dishes, and its flavor is reminiscent of shrimp or dwarf goatfish. Regarding the utilization of the stipes in recipes, most informants reported that they possess a very fibrous texture and because of that they are discarded or fried into chips for consumption (Figure 24H). A summary of the cooking methods and their frequencies employed in the preparation of *Macrolepiota* mushrooms according to the respondents are illustrate in Figure 26.

Some respondents store mushrooms by drying and/or pickling, although they prefer to consume them fresh. The flavor and tenderness of younger basidiomata were reported to be superior, while overmature mushrooms can taste unpleasantly bitter when cooked. For this reason, some informants opt to dry and subsequently pulverize overmature mushrooms for their utilization as a seasoning.

Figure 23. *Macrolepiota* mushrooms harvested and prepared by informants in the states of Rio Grande do Sul (**A**, **D**) and Minas Gerais (**B**–**C**), Southern and Southeastern Brazil, respectively. **A**. *Macrolepiota abruptibulbosa*. **B**–**C**. *Macrolepiota chapeleta*. **D**. *Macrolepiota* cf. *bonaerensis* **E**–**I**. Records made by two informants from Southern Brazil, documenting the preparation of *Macrolepiota* mushrooms for consumption. **E**, **F**. *Macrolepiota* cf. *bonaerensis*. **E**. Cap grilling on a barbecue. **F**. Cap after grilling. **G**–**I**. *Macrolepiota abruptibulbosa*. **G**. Entire cap before preparation. **H**. Cap slices sautéed in butter. **I**. Cap sautéed slices accompanied by spaghetti in pesto sauce. These photographs were made available by the informants, with their kind permission. Photographs: **A**, **G**–**I**. Helissa Gründemann's personal archive. **B**–**C**. Thiago V. D. H. Comunale's personal archive. **D**–**F**. Jeferson Müller Timm.

Figure 24. A participant observation record of a Polish-Brazilian farmer from the initial stages of harvesting wild *Macrolepiota* mushrooms to the subsequent preparation of the mushrooms at home, Parelheiros district. **A**. *Macrolepiota bonaerensis* in the field. **B**–**C**. Harvested basidiomata. **D**–**E**. Separation of the caps and the stipes before cooking. **F**. Mushroom caps being sautéed in butter. **G**–**H**. Frying and fried stipes, respectively. **I**. Sautéed caps with bread. Photographs: Amanda Prado-Elias.

Marketability – from the woods to the kitchen

Other than food service, some of the *Macrolepiota* mushrooms from the Parelheiros district are used as an alternative to supplement household income (Figure 27). During the summer and fall months (December to April), at least two key informants indicated involvement

in the sale of *Macrolepiota* mushrooms and some other WEM, including *Lactarius quieticolor*, *Agaricus* spp., *Collybia sordida* (field Blewit), and *Laetiporus gilbertsonii* (chicken of the woods). These informants are officially registered as smallholder extractive farmers and have reported that up to 2.5 kg of wild *Macrolepiota* fresh mushrooms are harvested per each foraging trip. During a participant observation, two types of *Macrolepiota* specimens were informally recognized as good for sale. These specimens were identified by the informants as representing two different species: (1) a pale/white species with a minutely squamulose pileus that thrives in pastures and other grassy habitats; and (2) a brownish to brownish-orange species with adnate zigzag bands on stipe surface, found in large numbers on the outskirts of native forest areas. Morphological and molecular analyses of collections corresponding to each of these morphotypes revealed the presence of three distinct species, namely *M. bonaerensis*, *M. capelariae*, and *M. chapeleta*. The first corresponds to the pale species found in pastures (species 1, according to the informants), while the other two species were associated with native forest areas (species 2, according to the informants).

Most harvested species of *Macrolepiota* are sold fresh, while dried mushrooms are mainly for household consumption. The price per kg varies from $100 to $120 Brazilian reais (BRL) (~ $20–25 USD, $1 USD ≈ $5 BRL). The distribution network for these fresh mushrooms includes supplying the kitchens of some restaurants situated in São Paulo's downtown area, such as AE Cozinha, Chef Rouge, Charco, Banana Verde, and 31. The latter restaurant was identified as the primary purchaser of the local WEM and its culinary is focused on plant-based haute cuisine, incorporating locavorism and the utilization of seasonal ingredients from Brazilian biodiversity. *Macrolepiota* mushrooms, according to the 31 restaurant's chef, are typically prepared by grilling at a low temperature, which ensures a texture reminiscent of oysters and a delicate and distinctive flavor (Figure 25F). Furthermore, they are used in the preparation of risottos (Figure 25E).

Figure 25. Dishes prepared with wild *Macrolepiota* mushrooms that were cooked during the focus group discussions or provided by the participants during the interviews. **A**. "Picadinho" – pieces or whole mushroom caps browned in olive oil and then cooked in some liquid and carrot slices in a closed pan. **B**. Detail a mushroom cap from the dish shown in **A**. **C**. Mushroom caps sliced and sautéed in butter with handmade "taioba" (tannia) ramen. **D**. Focaccia with sliced mushroom caps, onion, and rosemary. **E**. Risotto with whole basidiomata (*M. bonaerensis* and *L. quieticolor*). **F**. Grilled basidioma of *M. bonaerensis* with fleur de sel, a bite-sized dish from the autumn tasting menu at Restaurant 31. **G**. Raw *M. capelariae* basidiomata seasoned with curry. **H**. Risotto prepared with *M. bonaerensis* and a few young basidiomata of *M. capelariae*. Photographs and cooking: **A**–**D**. Neide Rigo. **E**–**F**, **H**. Raphael Vieira, chef from Restaurant 31, in São Paulo downtown. **G**. Thiago V. D. H. Comenale.

Figure 26. Frequency of cooking and storage methods used for harvested *Macrolepiota* species according to the 22 respondents in the interviews.

Figure 27. Smallholder extractive farmers engaged in mushroom foraging in the Parelheiros district of São Paulo city, Southeastern Brazil. These photographs were made available by the informants, with their kind permission Photographs: **A**−**B**. Cristiano Coelho-Nascimento. **C**−**E**. Bruno Henrique da Silva's personal archive.

Nutritional Composition

Proximate nutritional value and mineral profile of whole basidiomata of *M. bonaerensis* (NCC289), *M. capelariae* (NCC291), and *M. chapeleta* (NCC294) are provided in the following sections.

Proximate nutritional value

The proximate compositions (% dry weight − DW) and estimated energy values obtained for the previously mentioned *Macrolepiota* species are shown in Table 3. For comparative purposes, Table 3 also includes macronutrient data from the published literature for the three most important cultivated mushrooms worldwide (viz., *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus*) and for wild mushroom species commonly found in South America (viz., *Auricularia cornea*, *Favolus brasiliensis*, and *Oudemansiella cubensis*). In addition, Figure 28 presents data regarding the macronutrient composition of fresh-harvested basidiomata, expressed on a wet weight (WW) basis, for the three *Macrolepiota* species examined in this study.

Water has the largest share in the fresh mushrooms, an indication of their high susceptibility to microbial contamination and spoilage but also acting to provide flavor and texture (Mahajan *et al*. 2008, Krishnamoorthi *et al*. 2022, Effiong *et al*. 2024). The moisture content (% fresh weight − FW) was 83,63% in *M. bonaerensis*, 84,41% in *M. capelariae*, and 84,77% in *M. chapeleta*; thus, by difference, the dry matter represents 15,23–16,37%. These results are in consonance with range of 82.0–87.1% assumed for fresh mushrooms (Mirończuk-Chodakowska & Witkowska 2020). However, it should be noted that the moisture content reported for important cultivated mushrooms such as *A. bisporus*, *L. edodes*, and *P. ostreatus* was typically found to exceed 90% (Mau *et al*. 2021, Usman *et al*. 2021, Effiong *et al*. 2024).

Carbohydrates are one of the primary nutrients in mushrooms and constitute approximately 35–75 % DW (Crisan & Sands 1978, Adamska and Tokarczyk 2022). Non-fiber carbohydrates were the most abundant constituents (48.37–50.26%) in all of the studied mushrooms (**Table 3**). This result is in correlation with the 37.57–56.2% reported for *M.*

dolichaula (Kumari & Atri, 2014, Rizal *et al.* 2015) and the 51.58–55.80% reported for *M. mastoidea* (Colak *et al*. 2007, Ćirić *et al.* 2019). However, the carbohydrate content of 60.3–80.38% reported in some studies for *M. procera* was considerably higher than that observed for the herein evaluated *Macrolepiota* species (Fernandes *et al*. 2012, Kumari & Atri 2014, Ćirić *et al.* (2019). Distinctly variation in these outcomes might be caused by environmental conditions, given that *M. procera* is regarded as a common species with a wide distribution throughout Eurasia. Furthermore, it should be noted that this taxon is currently the most extensively evaluated in terms of nutritional value among edible *Macrolepiota* representatives (Adamska and Tokarczyk 2022).

Table 3. Proximate nutritional composition of *M. bonaerensis*, *M. capelariae*, *M. chapeleta*, and sampled species derived from the scientific literature. Values are expressed in dry weight basis percent and presented as μ ± σ of three replicates. The values presented in boldface were generated in the present study.

Species	Energy (Kcal/100 g)	Ash	Crude Protein	Carbohydrates	Crude fibre	Crude fat	Reference
***Macrolepiota* species**							
M. bonaerensis	**339.20 ± 2.23**	**8.83 ± 0.30**	**30.65 ± 0.11**	**48.37 ± 0.53**	**15.86 ± 0.13**	**2.61 ± 0.01**	**--**
M. capelariae	**355.09 ± 1.06**	**8.37 ± 0.07**	**32.27 ± 2.50**	**50.26 ± 2.20**	**14.34 ± 0.25**	**2.77 ± 0.07**	**--**
M. chapeleta	**342.05 ± 1.86**	**8.13 ± 0.35**	**35.48 ± 1.25**	**44.81 ± 0.78**	**14.73 ± 0.37**	**2.34 ± 0.21**	**--**
M. dolichaula	333.40 ± 0.11[1]	7.30 ± 0.15	19.95 ± 1.35	56.20 ± 0.10	4.85 ± 0.18	3.20 ± 0.20	**A**
M. dolichaula	285.60 ± 0.07[1]	10.19 ± 0.59	27.36 ± 0.08	37.57 ± 0.08	15.69 ± 0.42	1.59 ± 0.16	**B**
M. mastoidea	368.37 ± 0.01	12.44 ± 0.03	32.36 ± 0.05	51.58 ± 0.01	--	3.62 ± 0.03	**C**
M. mastoidea	397.66 ± 14.30	5.40 ± 0.40	33.31 ± 3.00	55.80 ± 6.00	--	4.58 ± 0.30	**CC**
M. procera	345.70 ± 12.30	6.80 ± 0.10	26.35 ± 1.50	54.70 ± 2.80	--	2.40 ± 0.20	**D**
M. procera	365.01± 0.59	9.86 ± 0.72	7.62 ± 0.08	80.38 ± 0.19	--	1.45 ± 0.13	**E**
M. procera	353.70 ± 0.06[1]	1.93 ± 0.06	19.95 ± 1.06	60.82 ± 0.11	5.10 ± 0.22	3.40 ± 0.08	**A**
M. procera	--	15.50 ± 0.10	9.80 ± 0.10	30.50 ± 0.20	13.50 ± 0.10	11.5 ± 0.20	**F**
M. procera	375.70 ± 0.40	8.50 ± 0.10	29.20 ± 0.50	60.30 ± 0.20	--	1.90 ± 0.20	**C**
Commercially important cultivated mushrooms							
A. bisporus	334.80[1]	--	29.14	51.05	--	1.56	**G**
A. bisporus	377.40[1]	11.40	22.70	61.30	27.50	4.60	**H**
A. bisporus	--	10−10.10	26.50−27.10	58.40−59.50	19.50−20.50	4−4.30	**I[2]**
A. bisporus	372.10[1]	13.10	33.30	48.70	32.80	4.90	**J**
A. bisporus	--	11.20−12.70	28.40−40.80	43.30−57.60	7.80−17	1.30−4.50	**K[3]**
A. bisporus	317.68[1]	7.77 ± 0.21	21.70 ± 0.40	48.90 ± 0.51	17.70 ± 0.71	3.92 ± 0.71	**L**
A. bisporus	345.10[1]	8.70 ± 1.80	32.10 ± 3.60	47.20 ± 4.70	8.90 ± 2.00	3.10 ± 0.60	**M**
A. bisporus	336	9.30	25.10	52.70	2.90	1.40	**N**
L. edodes	340.78[1]	--	18.85	63.60	--	1.22	**G**
L. edodes	--	3.22−5.08	12.94−21.07	54.19−64.78	3.27−4.01	1.42−2.24	**O[2]**
L. edodes	--	7.83−10.39	23.44−25.87	63.83−64.36	--	1.80−2.13	**P[2]**
L. edodes	340	6.10	20.70	59.50	3.80	1.50	**N**
L. edodes	--	5.18−5.20	26.80−28	33.90−37.40	27.90−28.30	3.90−5.30	**Q[2]**
L. edodes	--	5.26−6.50	13.67−19.60	52.17−59.39	12.99−18.00	0.88−1.83	**R[5]**
L. edodes	--	5.12−7.75	16.01−22.44	49.25−64.23	4.04−7.69	2.00−3.97	**S[4]**
P. ostreatus	342.51−394.57	4.28−6.32	9.83−11.27	70.02−77.56	3.09−3.98	4.40−5.72	**T[2]**
P. ostreatus	346.90[1]	7.7 ± 1.60	20 ± 3.10	61.10 ± 4.20	7.90 ± 2.10	2.50 ± 0.70	**M**
P. ostreatus	354.80 ± 0.04	9.14	25.15	66.54	7.43	1.75	**U**
P. ostreatus	336,81[1]	8.17 ± 0.99	18.36 ± 1.61	56.28 ± 1.90	9.09 ± 1.23	4.25 ± 1.28	**V**
P. ostreatus	286,58	8.22 ± 0.04	17.06 ± 0.17	43.42 ± 0.01	23.63 ± 0.01	1.21 ± 0.02	**W**
Common wild edible mushrooms from South America							

A. cornea	422.15[1]	4.24	11.59	71.02	19.63	10.19	**X**
F. brasiliensis	--	1.70	27.00	--	17.00	1.50	**Y**
O. cubensis	270	8.60 ± 0.11	25.52 ± 0.50	12.30 ± 1.00	38.04 ± 0.00	13.19 ± 0.74	**Z**
O. cubensis	--	10.65 ± 0.07	11.50 ± 0.30	23.72 ± 0.55	32.35 ± 0.45	10.45 ± 0.03	**AA**

Notes: **A** = Kumari & Atri (2014), **B** = Rizal *et al.* (2015), **C** = Ćirić *et al.* (2019), **CC** = **C**olak *et al*. (2007), **D** = Ayaz *et al*. (2011), **E** = Fernandes *et al*. (2012), **F** = Adebola *et al*. (2016), **G** = Ahlawat *et al*. (2016), **H** = Manzi *et al*. (2001), **I** = Mattila *et al*. (2002), **J** = CSTJ (2005), **K** = USDA (2005), **L** = Tsai *et al*. (2007), **M** = Shbeeb *et al*. (2019), **N** = Dimopoulou *et al*. (2022), **O** = Kim *et al*. (2017), **P** = Alberti *et al*. (2022), **Q** = Rahman *et al*. (2023), **R** = Desisa *et al*. (2024), **S** = Paswal *et al*. (2024), **T** = Okon & Udoakah (2015), **U =** Irshad *et al*. (2023), **V** = Boadu *et al*. (2023), **W** = Effiong *et al*. (2024), **X** = Drewinski *et al*. (2024), **Y** = Silva-Neto (2021), **Z** = Mancuello *et al*. (2024), **AA** = Alberti *et al*. (2021).
[1] Energy value first calculated or recalculated according to Kumari & Atri *et al*. (2014) methods; **[2]** Data from different *A. bisporus*, *L. edodes* or *P. ostreatus* strains; **[3]** Data from different strains and growth stages of *A. bisporus*; **[4]** Data from different *L. edodes* strains grown on different substrates; **[5]** Data from a *L. edodes* strain grown on different substrates.

In recent years, protein content has been a leading trend in food growth (Vallath *et al*. 2022). A rising demand for non-animal protein sources has led to an crescent interest in plant and fungal protein (Vallath *et al*. 2022, Wang *et al*. 2023). The evaluated *Macrolepiota* species presented significantly high protein content, 30.65–35.48%. These results are consistent with the reported values of 32.36–33.31% for *M. mastoidea* (Colak *et al*. 2007, Ćirić *et al.* 2019) and contrasts with the sampled values for *M. dolichaula* and *M. procera*, which exhibited lower protein contents, 7.62–29.2% (Table 3). Notably, for popular cultivated mushrooms, as well as for common wild edible mushrooms from South America, lower protein contents were observed for all sampled species, 9.83–29.14% (Table 3), with the sole exception of the 32.1% reported by Shbeeb *et al*. (2019) for *A. bisporus.* Furthermore, given that edible mushrooms contain, on average, 19%–35% protein by DW (Wani *et al*. 2010), the present results demonstrate that *M. bonaerensis*, *M. capelariae*, and *M. chapeleta* can serve as a valuable protein source. They provide protein values that are higher than or comparable to common plant or animal derived food items such as legumes (22–40%), cereals (8–18%), nuts (4–20%), milk (25.2–27%), rice (7.3%), wheat (13.2%), and soybean (39.1%) (Alam *et al*. 2008, Butnariu & Butu 2014, Floret *et al*. 2021, Desisa *et al*. 2024).

Mushrooms are low in fat, cholesterol-free, and have a high percentage of unsaturated fatty acids required by the body (Wang & Zhao 2023). They typically have a crude fat content of 1–15% by DW (Sande *et al*. 2019). The fat content percentage of the investigated *Macrolepiota* species were of 2.34–2.77%. A distinctly low-fat content was also observed in all values reported for *M. dolichaula*, *M. mastoidea*, and *M. procera* that ranged from 1.59% to 4.58%, with the exception of the considerably higher value of 11.5% reported by Adebola *et al*. (2016) for latter species (Table 3). Furthermore, the low crude fat level observed in edible *Macrolepiota* species is directly correlated with their low-energy content (285.6–397.66 Kcal, Table 3), indicating their value as a health food, especially with respect to their potential for obesity management.

Dietary fiber has various biological activities and are a crucial component of foods that can benefit health (Yu *et al*. 2023). Mushrooms are rich in dietary fiber and their fibrous structure also makes the taste closer to real meat (Kumar *et al*. 2017). The crude fiber content obtained in this study were of 14.34–15.86%. These values are consistent with the reported values of 15.69% and 13.5% for *M. dolichaula* and *M. procera*, respectively (Rizal *et al.* 2015, Adebola *et al*. 2016). However, contrasting lower values (4.85% and 5.1%, respectively) were also observed for these species (Kumari & Atri 2014) (Table 3). It is worth noting that the consumption of the evaluated *Macrolepiota* species as part of a daily diet can easily provide about 25% of the recommended dietary intake of dietary fiber (Cheung 2013).

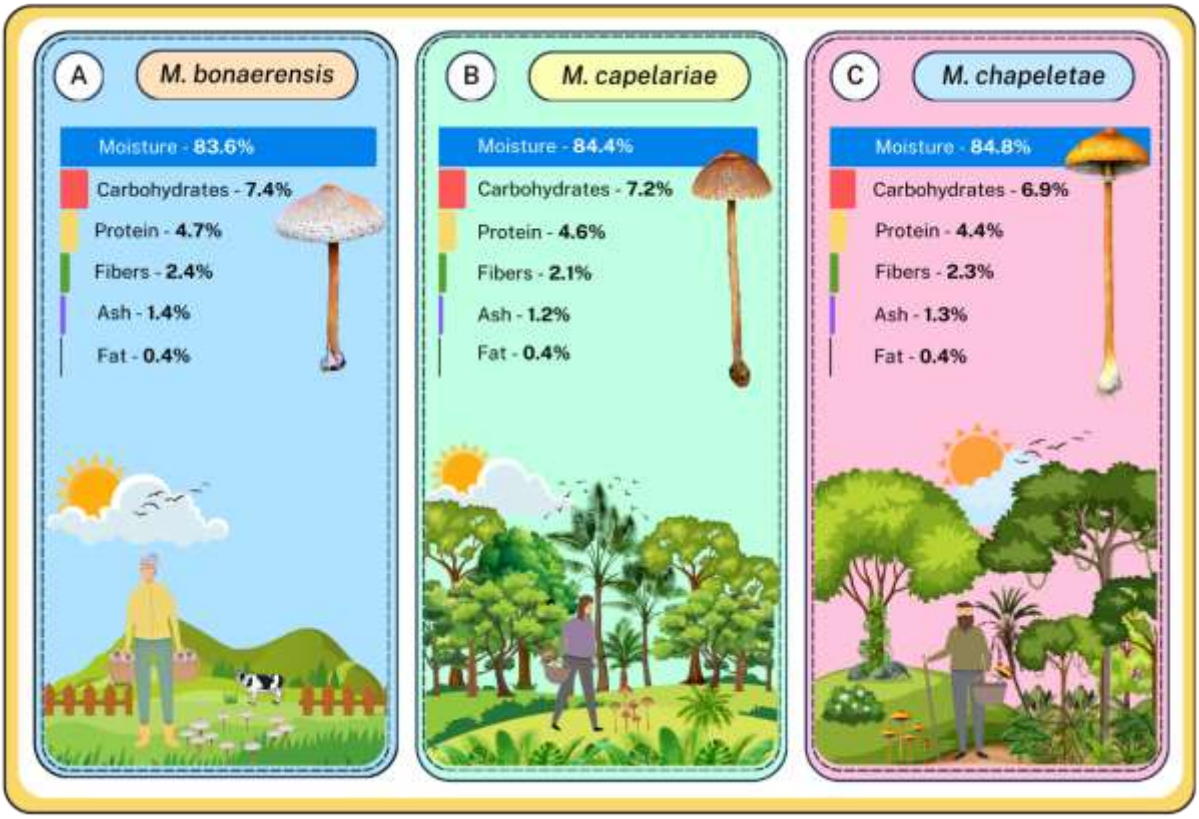

Figure 28. Proximate nutritional composition (% wet weight) of fresh-harvested *Macrolepiota* mushrooms and graphical illustration of their associated habitats in the Parelheiros district of São Paulo city, Southeastern Brazil. **A**. *Macrolepiota bonaerensis*. **B**. *Macrolepiota capelariae*. **C**. *Macrolepiota chapeleta*.

Ashing procedures are available to concentrate the sample and rid it of unwanted organic matrix, and to retain an inorganic residue, referred to as ash (Casey *et al*. 1995). The ash content directly reflects the presence of important minerals (Yu *et al*. 2020), representing approximately 7–19% DW in mushrooms (Dogan *et al*. 2022). Ash content among the studied *Macrolepiota* species exhibited minimal variability, ranging from 8.13 to 8.83% DW. These

values are consistent with the values most frequently reported for *M. dolichaula*, *M. mastoidea*, and *M. procera*, 5.4–10.19% (Table 3). It is important to note, however, that a few of the reported ash values for *M. procera* are found to be significantly divergent from the majority of the reported values (Table 3). For example, studies conducted by Kumari and Atri (2014), Ćirić *et al.* (2019) and Adebola *et al*. (2016) reported ash contents of 1.93%, 12.44, and 15.5%, respectively. In comparison, the ash content of important cultivated species exhibited a narrower range, 3.22%–12.7% (Table 3). It is reasonable to anticipate that greater variations in ash content occur in wild-growing species than in cultivated ones, probably due to the more variable substrates in the former group (Kalač 2012). Additionally, variations in the mineral composition across different parts of the mushrooms (stems and caps) and the correlation between their content and the mushroom maturity stage have also been observed (Adamska and Tokarczyk 2022).

Macrominerals Profile

The macrominerals contents of the studied samples are given in the Table 4 along with recommended daily intake (RDI) for each element. All concentrations were determined on a DW basis and expressed in milligrams per 100 grams (mg/100 g). Concentration values from the literature were converted to this unit where necessary in order to facilitate comparison.

The evaluated *Macrolepiota* species can be considered as relatively rich in K, P, Mg, and Ca (Table 4). Among these essential macrominerals, potassium (K) was found in highest amounts, ranging from 1931.67 to 2003.33 mg/100g, with the highest concentration observed in *M. capelariae*. In literature, the K levels of *Macrolepiota* species have been previously investigated exclusively in *M. procera*, a species characterized by a high potential to bioconcentrate K (Adamska and Tokarczyk 2022). The K content of *M. procera* was found to exhibit a total range from 980 to 5700 mg/100g (Ayaz *et al*. 2011, Uzun *et al*. 2011, Gucia *et al*. 2012A, 2012B, Kuldo *et al*. 2014, Adebola *et al*. 2016, Kojta *et al*. 2016, Senila *et al*. 2024).

Table 4. Composition of minerals in wild mushrooms of *M. bonaerensis*, *M. capelariae*, and *M. chapeleta* with the recommended daily intake for each element. The levels of each mineral are expressed as mg/100 g of mushroom. Values are μ ± σ of three replicates.

Mineral	**Concentration (mg/100 g DW)**			**Recommended daily intake* (mg)**
	M. bonaerensis	***M. capelariae***	***M. chapeleta***	
Macrominerals				
Potassium (**K**)	**1931.67** ± 34.7	**2003.33** ± 78.5	**1946.67** ± 41.1	3500

Magnesium (**Mg**)	**132** ± 1.63	**115** ± 4.08	**92** ± 1.63	350–400
Phosphorus (**P**)	**1383.33** ± 28.6	**983.33** ± 24.9	**1136.67** ± 57.9	1000
Calcium (**Ca**)	**63.33** ± 3.40	**23.33** ± 2.87	**32.67** ± 2.49	1000
Microminerals				
Iron (**Fe**)	**28.13** ± 0.39	**12.55** ± 0.55	**17.13** ± 0.66	15–18
Manganese (**Mn**)	**1.05** ± 0.15	**0.36** ± 0.04	**0.45** ± 0.04	5
Copper (**Cu**)	**22.87** ± 0.84	**24.2** ± 0.65	**26.63** ± 0.97	2
Zinc (**Zn**)	**9.53** ± 0.34	**8.67** ± 0.29	**11.93** ± 0.74	15

*****Recommended daily intake [Sources: FAO (2001), Otten *et al*. (2006)].

Potassium content in the evaluated species is followed by Phosphorus (P), Magnesium (Mg), and then Calcium (Ca). The P, Mg, and Ca levels exhibited higher levels in the *M. bonaerensis* (1383.33, 132 and 63.33 mg/100 g, respectively) when compared to the *M. capelariae* (983.33, 115 and 23.33 mg/100 g, respectively) and *M. chapeleta* (1136.67, 92 and 32.67 mg/100 g, respectively) (Table 4). As with the K contents, the literature provides P levels exclusively for *M. procera*, ranging from 470 to 1800 mg/100 g (Gucia *et al*. 2012A, 2012B, Kuldo *et al*. 2014, Senila *et al*. 2024), while Mg and Ca levels have been detected in both *M. procera* (50–330 and 6.8–410 mg/100 g, respectively, Ayaz *et al*. 2011, Uzun *et al*. 2011, Gucia *et al*. 2012A, 2012B, Kuldo *et al*. 2014, Kumari and Atri 2014, Kojta *et al*. 2016, Senila *et al*. 2024) and *M. dolichaula* (0.99–143 and 0.34–5 mg/100 g, respectively, Kumari and Atri 2014, Rizal *et al.* 2015).

The elements treated in this section are essential to fungus and their up-take and sequestration in the basidiomata can be regulated by a species (Gucia *et al*. 2012B). Notwithstanding, it is known that the chemical composition of the substrate significantly affects the chemical composition of the mushrooms (Adamska and Tokarczyk 2022). Furthermore, differences in the content of various elements in individual parts of the mushrooms (stipes and caps) and the dependence of their content on the age of the fruiting body were also observed (Adamska and Tokarczyk 2022).

Microminerals Profile (Essential trace metals)

Trace metals of nutritional value [Iron (Fe), Manganese (Mn), Copper (Cu), and Zinc (Zn)] were identified at relatively high concentrations for the three edible *Macrolepiota* species studied, with the exception of Mn content, for which low concentrations (0.81–1 mg/100 g) were observed (Table 4). This result is in correlation with the median concentrations of essential trace metals reported by Gucia *et al*. (2012A) for *M. procera* specimens collected from northern Poland (Fe: 3.2–48 mg/100 g, Mn: 1.1–5.5 mg/100 g, Cu: 6.1–21 mg/100 g, and Zn: 4.6–19

mg/100 g). In terms of the bioconcentration/exclusion concept, the latter study found that Cu and Zn were highly and moderately bioconcentrated, respectively, while Fe and Mn were excluded. A similar profile involving the concentration/exclusion of essential trace metals in *M. procera* emerged from the study by Gucia et al. (2012B) based on specimens from northern Poland, with Fe: 7.7–24 mg/100 g, Mn: 1.5–3 mg/100 g, Cu: 9.1–18 mg/100 g, and Zn: 7.4–14 mg/100 g. In contrast, Kumari and Atri (2014) observed a conspicuously high Fe content (276 mg/100 g) in *M. procera* specimens from India, while Mn, Cu, and Zn were found at low levels (5, 9, and 0.06 mg/100 g, respectively). Kumari and Atri (2014) also examined the microminerals present in *M. dolichaula* from the same area, and the concentrations observed were analogous to those detected in *M. procera*, with Fe: 241 mg/100 g, Mn: 1 mg/100 g, Cu: 5 mg/100 g, and Zn: 0.08 mg/100 g. Rizal et al. (2015) subsequently detected significantly lower levels of Fe in specimens of *M. dolichaula* cultivated in Thailand, although the content of Mn, Cu, and Zn found was similarly low, with Fe: 15.94 mg/100 g, Mn: 0.81 mg/100 g, Cu: 1.94 mg/100 g, and Zn: 1.69 mg/100 g.

Finally, it is also worth drawing attention to the fluctuations in the concentration of essential trace metals in *Macrolepiota* species evidenced by the contrasting levels of Fe in different collections of *M. procera* and *M. dolichaula*, as discussed in this section. This suggests that a particular species may be able to bioconcentrate or exclude certain microminerals depending on the pedochemical conditions of its place of origin, which ultimately reflects the amount of these metals present in the soil (Gucia *et al*. 2012a, b).

DISCUSSION

Taxonomic novelties and improvements

This study is the initial milestone of an integrative taxonomic research of *Macrolepiota* from the Americas including morphological, molecular, and ecological data to species delimitation, as well as incorporating ethnomycological knowledge to recognize edible species. Three new species and two new varieties of *Macrolepiota* are here described, in addition to the four new species described from Brazil in last decade by Fazolino Perez *et al*. (2018), Freitas & Menolli (2019), and Souza *et al.* (2022). Furthermore, we improved the taxonomy of old Neotropical species by stabilizing usage of the name *M. bonaerensis* and its now-established synonym *M. kerandi*. Nevertheless, classical studies still include old names in need of morphological/phylogenetic assessment. This is exemplified by *M. brasiliensis* and *M. stercoraria* from Southern Brazil, whose identity and autonomy relative to confirmed species

remain unresolved, mainly because of their known morphological traits are more in line with the generic concept of *Chlorophyllum* than with that of *Macrolepiota* (Rick 1907, 1939, 1961, Raithelhuber 1988, 1991). Finally, other sequenced species (viz. *M. cyanolamellata* and *M. sabulosa*) previously recovered within *Macrolepiota* sect. *Macrolepiota* (Fazolino Perez *et al*. (2018) are now shown to belong to *Macrolepiota* sect. *Velosa*, which seems to represent a more natural arrangement to these species and is congruent with the assumed section synapomorphy represented by the presence of a volva at the stipe base (Ge *et al*. 2010).

Comparisons of the single-locus and multi-locus phylogenies

Our molecular phylogenetic analyses (Figures 2, 3) show that the relationship resolution increases with the number of gene fragments employed. The ITS dataset is capable to delimit and recognize species in *Macrolepiota* as demonstrated by previous studies (Fazolino Perez *et al*. 2018, Freitas & Menolli 2019, Souza *et al.* 2022, Sysouphanthong *et al*. 2021, Lebeuf *et al*. 2024), and it is even good at recognizing some monophyletic groups (Figure 3). For example, ITS phylogenetic analyses provided insights into phenotypic variation and geographic distribution of *M. capelariae* (Souza *et al.* 2022). However, our phylogenetic results suggested that the resolution of internal topology and some interspecific relationships based solely on ITS sequences is insufficient. Although ITS sequences are important in species delimitation of *Macrolepiota*, they are not adequate to fully resolve the infrageneric phylogenetic relationships. On the other hand, our four-locus dataset (ITS-nrLSU-*rpb2*-*tef1*-*α*) was able to better resolve section-level phylogeny, as well as improving the support for internal branches. Nevertheless, it is worth noting, in this respect, that few sequences of both nrLSU and protein-coding markers (viz. *rpb2*, *tef1*-*α* etc.) are available in public repositories for *Macrolepiota*, even for well-sampled regions such as Europe, where the genus has already received a monographic treatment (Candusso & Lanzoni 1990). This current lack of information prevents deeper insights into the intrasectional relationships and the better elucidation of cryptic and pseudo-cryptic species.

The congruence of phylogeny with morphology in the three sections of Macrolepiota

The systematics in *Macrolepiota* has been based on morpho-anatomical and ecological features, whose taxonomic value can now be objectively evaluated in light of DNA phylogenies. Not surprisingly, perhaps, many traits listed by Ge *et al*. (2010) as characteristic of each section need to be re-evaluated based on a more robust phylogeny that virtually includes well-described representatives from all continents. At this point, we will discuss the common

morphological characters assigned to each section by Ge *et al*. (2010) in relation to our improved phylogeny.

The clade /Volvatae, corresponding to *Macrolepiota* sect. *Volvatae*, represents an independent evolutive unit mainly distributed in tropical regions (Vellinga 2003, Vellinga & Yang 2003). It has been defined by species having a volvate stipe base, finely squamulous stipe surface, relatively small amygdaliform-ellipsoid basidiospores, and no clamp connections (Ge *et al*. 2010). Excluding the formation of a volva and a finely squamulous stipe surface, the other characters mentioned must be interpreted with caution as they do not constitute consistent taxonomic criteria to define species in this clade. The relatively small basidiospores (less than 15 μm in length) are shown to be a feature shared by all known species in this section, with exception of *M. cyanolamellata* that forms longer basidiospores (9.0–17.0 × 6.0–9.0 μm), albeit at a low frequency (Fazolino Perez *et al*. 2018). Concerning basidiospore shape, strictly amygdaliform-ellipsoid cannot be viewed as a consistent character because elongate to cylindrical basidiospores are common in species from the Neotropics within the /Volvatae clade (viz. *M. cyanolamellata*, *M. pulchella*, and *M. sabulosa*). Lastly, the absence of clamp connections, which is a common trait throughout species within the /Volvatae clade, should not be emphasized as synapomorphy because: (1) it is different in *M. cyanolamellata* and *M. sabulosa* that have common clamped hyphae at the stipe context and hymenium; and (2) it is also common in species outside *Macrolepiota* sect. *Volvatae*. It is also relevant to note that subsequent to the characterization of the sections proposed by Ge *et al*. (2010), a new volvate variety, namely *M. rhodosperma* var. *velicopia*, was described from Italy (Vizzini *et al*. 2011). Interestingly, this taxon was phylogenetically recovered within the non-volvate *Macrolepiota* sect. *Macrolepiota*, prompting the latter authors to conclude that the velar structure acquisition or loss is a homoplastic character that developed independently during the evolution of *Macrolepiota*, which makes it unsuitable for a natural classification of these fungi. However, we interpret that *M. rhodosperma* var. *velicopia* should not be regarded as a typical volvate species, given that the remaining universal veil fails to assume a distinctive limbate or peronate structure at stipe base as observed in representatives of the *Macrolepiota* sect. *Volvatae*; instead, it develops as small membranous patches on the bulb margin, with most of velar remnants left as patches on the pileus surface (Vizzini *et al*. 2011). The same can be observed in *M. capelariae* var. *velana*, which also presents small velar remnants at the stipe base and pileus surface and assumes a consistent position within *Macrolepiota* sect. *Macrolepiota*. In light of these insights, we advocate that the distinctive formation of a limbate or peronate volva in species of *Macrolepiota* sect. *Volvatae* should be regarded as an exclusive trait of this section.

The clade /Macrolepiota, corresponding to *Macrolepiota* sect. *Macrolepiota*, is the most speciose within the genus, encompassing species that are distributed on all continents, except Antarctica. Previously, from the Neotropics, only *M. capelariae* and *M. colombiana* have been recognized in this section. The current findings have identified an even greater diversity, which has been demonstrated to form two well-supported sister lineages (Figure 2). Furthermore, this study has shed light on an unnamed collection from North America that most likely represents an undescribed species of this group (Figure 3).

Macrolepiota sect. *Macrolepiota* and the representatives of its correspondent clade have been characterized by comprise species with very large basidiomata, a complex annulus, common presence of clamp connections, mainly broadly clavate cheilocystidia, relatively big (usually 14–20 µm in length) ovoid-ellipsoid basidiospores, and a bilayered pileus covering with the terminal layer composed of brownish and thick-walled cylindrical hyphae (Ge *et al*. 2010). Indeed, the species in this section are notable for producing the largest basidiomata in the genus. An exceptional example is *M. chapeleta*, with reported maximum dimensions of 210 mm for the pileus diameter and 500 mm for the stipe length. It can be observed that certain species (e.g. *M. bonaerensis*) may exhibit shorter stipes that attain a length comparable to the pileus diameter. Nevertheless, these species are still relatively robust, mainly when compared to members of *Macrolepiota* sect. *Volvatae*. It is also evident that the relatively large basidiospores (≥ 14 µm on average in length) are a typical trait observed in the most species studied of *Macrolepiota* sect. *Macrolepiota*. Although they do not always adhere strictly to the oblong-ellipsoid shape, certain species, such as *M. chapeleta* and *M. capelariae*, exhibit commonly elongated to cylindrical basidiospores. The presence of clamp connections also appears to be a shared character of the species within *Macrolepiota* sect. *Macrolepiota*, although it must be evaluated with caution. Ge *et al*. (2010) reported the common occurrence of these elements at the base of basidia and cheilocystidia for species in this section. Nevertheless, in numerous collections of *M. chapeleta* and *M. capelariae*, clamp connections were either not found or extremely rare and only observed in the stipe context hyphae. Regarding the pileus covering, it is indeed accurate that all species of *Macrolepiota* sect. *Macrolepiota* form an intricate bilayered trichoderm (Ge *et al*. 2010). However, in contrast to what has been reported for Eurasian and Australian taxa (Ge *et al*. 2010, 2012), some Neotropical species lack the presence of strictly thick-walled and cylindrical elements in the terminal layer. Furthermore, elements elsewhere in the trichoderm are on average significantly broader, often producing conspicuously globose, broadly clavate, to widely oblong elements toward the basal zone. Finally, at the sectional scale, the shape of the cheilocystidia does not constitute a consistent taxonomic criterion, as it exhibits considerable variation among the

species of *Macrolepiota* sect. *Macrolepiota*. The mainly broadly clavate cheilocystidia reported by Ge *et al*. (2010) for this section are not commonly found among the species of *Macrolepiota* sect. *Macrolepiota* examined in the present study, nor among those recently described from North America by Lebeuf *et al*. (2024).

The clade /Macrosporae, corresponding to *Macrolepiota* sect. *Macrosporae*, is a residual lineage comprising a few known species worldwide (viz. *M. excoriata*, *M. mastoidea*, and *M. orientiexcoriata* Z.W. Ge, Zhu L. Yang & Vellinga) that seems to be absent or extremely rare in the Neotropics. The species in this clade are distinguished by a smooth stipe, a simple annulus, rare clamp connections, and a single-layered trichodermial pileus covering composed of rarely branched, thin-walled cylindrical hyphae (Ge *at al*. 2010). These diagnostic morphological traits remain a reliable means of defining species within this section, considering the absence of newly studied collections in *Macrolepiota* sect. *Macrosporae* since the efforts of Ge *et al*. (2010).

Firsts insights into ecology and distribution patterns of Macrolepiota species *from the Neotropics*

Ecological features such as geographical distribution and habitat are of paramount importance to species differentiation or identification in *Macrolepiota*. Range-restricted geographical distribution of *Macrolepiota* species has been frequently reported, with closely related species co-occurring in relatively small distribution areas, and few species having an extended distribution (Courtecuisse & Duhem 1994, Nauta & Vellinga 1995, Candusso & Lanzoni 1990, Vellinga 2004, Fiaz *et al*. 2014). Indeed, with the notable exception of *M. capelariae*, which occurs throughout the Neotropics (Souza *et al.* 2022), all the species dealt with in the present work seem to have a more restricted distribution. For example, *M. sabulosa* and *M. dunensis* have only been reported in the state of Rio Grande do Norte, Northeastern Brazil, where their occurrences probably depend on edaphic conditions of 'restinga', a tropical coastal vegetation of nutrient-poor sandy soil. Similarly, pedoclimatic conditions may have also contributed to the putative range-restricted occurrence of *M. abruptibulbosa* and *M. pernuda*; the first was exclusively found at relatively high altitudes (907–1.031 m a.s.l.) in pine forest areas, whereas the latter has so far been exclusively collected in upper montane forests in the 'Serra da Mantiqueira' area, Southeastern Brazil. It is worth noting that these distributional insights were based in the context of years of experience collecting and studying *Macrolepiota* specimens, mainly in Southeastern and Southern Brazil, as well as the constant monitoring of records in Neotropical areas using the iNaturalist platform. Nevertheless, it becomes apparent

that in the absence of a wide phylogenetic taxon sampling from poorly explored Neotropical areas, it is virtually impossible to draw reliable taxonomic conclusions on the ecological and distributional boundaries of the species addressed in this study. Therefore, it is crucial to invest both human effort and funding in supporting forays, sampling, and dedicated taxonomic studies. This should be done with a focus on biodiversity hotspots and neglected bioregions/ecoregions, such as those found in the Neotropics. By doing so, we can better understand and protect the diverse range of species that inhabit these areas.

Species limits in /colombiana clade

During this study, we came across an issue in defining species boundaries around the *M. colombiana* complex (clade /colombiana, Figures 2–3), because of the limited number of sequences available and the difficult task of translating DNA phylogenies into meaningful taxonomy. For example, pair *M. colombiana*/*M. pernuda* displayed very similar ITS sequences, genetically distinguishable by at least 5 SNPs and 1 indel. Unfortunately, determining the accurate evolutionary segregation events on the ITS locus between these two species is currently not feasible due to the unavailability of reference sequences for *M. colombiana* without a high proportion of ambiguous bases. Nevertheless, it is worth noting that several morpho-anatomical and ecological differences distinguish *M. pernuda* from *M. colombiana*, as discussed above. Similarly, *M. chapeleta* and *M. abruptibulbosa* from the sister clade /capelariae can be easily distinguished based on a number of morpho-anatomical and ecological traits, despite their high similarity at the ITS locus. Thus, *Macrolepiota* speciation events in the Neotropics are likely to be coupled with numerous early morphological changes, even in the presence of a small genetic divergence. Taking all of this into consideration, we have concluded that *M. pernuda* is indeed a 'good species', having a putative restricted biogeographical range and a very unique phenotype that clearly deviates from *M. colombiana*.

Ethnomycology

Over 400 wild edible mushrooms has been recorded from Brazil, including at least 86 consistent records based on molecular data and/or Brazilian nomenclatural types (Drewinski *et al*. 2024). However, traditional knowledge of consumption and use of these mushrooms was not exhaustively documented in Brazil (Trierveiler-Pereira & Prado-Elias 2022). Most ethnomycological studies are concentrated in Northern Brazil (Trierveiler-Pereira & Prado-Elias 2022), while only two studies (viz., Prado-Elias et al. 2022, 2024) that have investigated ethnomycological knowledge in local communities of Southern and Southeastern regions.

Regarding wild edible *Macrolepiota* mushrooms, no previous studies in Brazil have explored their traditional uses by local communities. For the other South American countries, only few ethnomycological studies have reported the uses of wild *Macrolepiota* species by local communities and indigenous peoples (Peña-Cañón & Enao-Mejía 2014, González-Cuellar et al. 2021, Bulla 2023). Of these studies, only Peña-Cañón & Enao-Mejía (2014) provided more detailed ethnomycological information, reporting the consumption of *M. colombiana* in peasant farmers communities associated with Andean oak forests in Colombia. In this context, the present study provides a pioneering account of the ethnomycological use of six wild *Macrolepiota* species in rural and semi-rural communities of Southern and Southeastern Brazil, namely *M. abruptibulbosa*, *M. bonaerensis*, *M. capelariae*, *M. chapeleta*, *M. pernuda* and *M. pulchella*. In essence, this study constitutes the first effort to document, delimit and describe the diversity of wild *Macrolepiota* species utilizing the broadest available range of information, integrating taxonomic and ethnomycological studies on a single front, and ultimately providing a practical taxonomic framework for both academic and non-academic communities alike.

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Capítulo 3

Unroughing the cat's tongue mushrooms: Four new species of *Pseudohydnum* from Brazil based on morphological and molecular phylogenetic evidence

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Unroughing the cat's tongue mushrooms: Four new species of *Pseudohydnum* from Brazil based on morphological and molecular phylogenetic evidence[6]

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ABSTRACT

Pseudohydnum, commonly known as cat's tongue mushrooms, is a monophyletic assemblage within Auriculariales, which encompasses species with gelatinous basidiomata, spathulate, flabellate, or shell-shaped pileus, hydnoid hymenophore, globose to ellipsoidal basidiospores, and longitudinally cruciate-septate basidia. According to the available literature, 16 species have been described in *Pseudohydnum*, mostly represented in temperate-boreal forests of the Northern Hemisphere. However, the limited morphological, molecular, and ecological information, especially from the Southern Hemisphere ecosystems, does not presently allow a reliable assessment of its taxonomic boundaries nor provide a complete picture of the species diversity in the genus. In an ongoing effort to examine specimens collected in dense and mixed ombrophilous forest fragments (Atlantic Rainforest domain) from Southeastern and Southern Brazil, additional taxa assigned to *Pseudohydnum* were identified. Four new species are recognized based mostly on characters of the pileus surface, stipe, hymenium, and basidiospores. Molecular phylogenetic analyses based on nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS barcode), partial nuc rDNA 28S, and partial RNA polymerase II largest subunit (RPB1) sequences supported the description of these new taxa. Here, we propose *Pseudohydnum brasiliense*, *P. brunneovelutinum*, *P. cupulisnymphae*, and *P. viridimontanum* as new species. Morphological descriptions, line drawings, habitat photos, and comparisons with closely related taxa are provided. A dichotomous key for identification of currently known Southern Hemisphere *Pseudohydnum* species is presented.

Keywords: Agaricomycetes; Auriculariales; jelly fungi; phylogeny; species diversity; 4 new taxa

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[6] Capítulo que originou o artigo "Unroughing the cat's tongue mushrooms: Four new species of *Pseudohydnum* from Brazil based on morphological and molecular phylogenetic evidence" publicado na Phytotaxa 09/08/2024 (https://doi.org/10.1080/00275514.2024.2363141) – Anexo B.

INTRODUCTION

Pseudohydnum P. Karst. is an enigmatic group of jelly fungi characterized by the stipitate to sessile gelatinous basidiomata, flabellate or shell-shaped pileus, hymenophore with sparse to crowded spines, globose to ellipsoidal basidiospores, and longitudinally cruciate-septate basidia (Bessette et al. 1996; Karsten 1868; Niveiro and Popoff 2011). The genus was originally introduced by Karsten (1868) within the order Tremellales because of these fungi's globose to ellipsoid basidia with longitudinal septa (Breitenbach and Kränzlin 1986) and later assigned to Auriculariales (Aporpiaceae) by Bandoni (1984), who redefined this order to accommodate heterobasidiomycetes with transversely septate basidia (auricularioid-type) as well as longitudinally septate basidia. This latter placement has been confirmed by some recent molecular phylogenetic studies, although *Pseudohydnum* has not yet been classified with certainty into a family (Chen et al. 2020; Spirin et al. 2023; Yuan et al. 2018).

Currently, only 16 species of *Pseudohydnum* have been described: (i) *Pseudohydnum abietinum* H.M. Zhou & Jing Si from northwestern China (Gansu Province) (Zhou et al. 2023), (ii) *P. alienum* Spirin & V. Malysheva from Russia (Karachay-Cherkessia, North Caucasus Region) (Spirin et al. 2023), (iii) *P. brunneiceps* Y.L. Chen, M.S. Su & L.P. Zhang from East China (Jiangxi Province) (Chen et al. 2020), (iv) *P. cystidiatum* V. Malysheva & V. Dudka from northwestern Vietnam (Cao Bằng Province) (Spirin et al. 2023), (v) *P. gelatinosum* (Scop.) P. Karst from western Slovenia (Inner Carniola) (Karsten 1868), (vi) *P. himalayanum* Y.C. Dai, F. Wu & H.M. Zhou from southwestern China (Yunnan Province) (Zhou et al. 2022), (vii) *P. meridianum* V. Malysheva & Spirin from central Vietnam (Gia Lai Province) (Spirin et al. 2023), (viii) *P. omnipavum* Spirin & Miettinen from western United States (Idaho state), (ix) *P. orbiculare* J.A. Cooper from New Zealand South Island (West Coast Region) (Zhou et al. 2022), (x) *P. placibile* V. Malysheva & V. Dudka from northwestern Vietnam (Cao Bằng Province) (Spirin et al. 2023), (xi) *P. sinobisporum* T. Bau, H.M. Zhou & Jing Si from northwestern China (Jilin Province) (Zhou et al. 2023), (xii) *P. sinogelatinosum* Y.C. Dai, F. Wu & H. M. Zhou from southwestern China (Yunnan Province) (Zhou et al. 2022), (xiii) *P. tasmanicum* Y.C. Dai & G.M. Gates from Tasmania, Australia (Zhou et al. 2022), (xiv) *P. totarae* (Lloyd) J.A. Cooper from New Zealand North Island (Wellington Region) (Zhou et al. 2022), (xv) *P. translucens* Lloyd from southern-central Japan (Lloyd 1925), and (xvi) *P. umbrosum* V. Malysheva & Spirin from Siberia, Russian Far East (Spirin et al. 2023). In addition, one subspecies, two forms, and two varieties of *P. gelatinosum* are recognized: *P. gelatinosum* ssp. *pusillum* (Ellis & Everh.) Miettinen & Viner from northwestern United States (Olympic Peninsula) (Spirin et al. 2023), *P. gelatinosum* f. *album* (Bres.) Kobayasi from Japan (Bresadola

1932; Kobayasi 1954), *P. gelatinosum* f. *fuscum* (Bres.) from Japan (Bresadola 1932; Kobayasi 1954), *P. gelatinosum* var. *bisporum* Lowy & Courtec. from French Guiana (Saut Pararé) (Courtecuisse & Lowy), and *P. gelatinosum* var. *paucidentatum* Lowy from western Bolivia (Nor Yungas Province) (Lowy 1959).

The type species of the genus, *P. gelatinosum*, commonly known as cat's tongue, false hedgehog mushroom, or white jelly mushroom, is considered edible and also a source of medicinal compounds, exhibiting anticancer, antibacterial, and antioxidant properties (Binion et al. 2008; Boulet 2003; Li et al. 2021; Sternisa et al. 2022; Wu et al. 2019). Although it was originally described from Europe, there are further regional treatments and reports, including North America (Volk et al. 1994), South America (Courtecuisse and Lowy 1990; Lowy 1971; Nivero and Popoff 2011), Central America (Lowy 1971; Mata et al. 2003), Asia (Dai 2010; Wu et al. 2009), and Oceania (McNabb 1964). Nevertheless, recent phylogenetic and diversity studies indicated that *P. gelatinosum* seems to be distributed only in temperate-boreal forests of the Northern Hemisphere, and its occurrence outside these areas is highly doubtful (Spirin et al. 2023; Zhou et al. 2022).

Despite some recent studies that employed molecular tools to unveil overlooked *Pseudohydnum* species (Chen et al. 2020; Spirin et al. 2023; Zhou et al. 2023, 2022), our knowledge of the genus' diversity and distribution is still incipient, especially in tropical and subtropical Southern Hemisphere forests, where extensive taxon sampling and molecular phylogenetic assessment of ambiguous collections are nonetheless lacking. Indeed, Brazilian taxonomic treatments of the genus are completely absent, except for a few reports of "*P. gelatinosum*" from the Amazon Forest (Bastos et al. 2015; Bononi 1981) and the description of *P. guepinioides* Rick from the Atlantic Rainforest (Rick 1904). The latter's protolog immediately evokes the concept of a stipitate *Trechispora* P. Karst species because of the cespitose, multilobate, cartilaginous-gelatinous basidiomata, and the ornamented spores (Rick 1904, 409); thus, Rick's taxon is considered a synonym of *T. thelephora* (Lév.) Ryvarden (Ryvarden 2002). The reports of "*P. gelatinosum*" from the Amazon Forest do not fit into any of the hitherto known species in the genus, likely referring to species yet to be described.

During our ongoing research on macrofungi focused on edible species, several specimens of *Pseudohydnum* were collected in Atlantic Rainforest fragments from Southern and Southeastern Brazil. The morphological examination and the molecular phylogenetic analyses for these collections revealed that they represent four new species. These species are formally described here as *P. brasiliense, P. brunneovelutinum, P. cupulisnymphae*, and *P. viridimontanum*. We accordingly present a morphological description of the new species

and also provide a dichotomous key for identification of currently known Southern Hemisphere *Pseudohydnum* species.

MATERIALS AND METHODS

Collection sites.—Sixteen specimens were collected from December 2019 to February 2023 in the Brazilian states of Espírito Santo, Paraná, Rio de Janeiro, and São Paulo. Collecting areas include the following Atlantic Rainforest protected reserves: Parque Estadual Campos do Jordão (PECJ), Parque Estadual da Cantareira (PEC), Parque Estadual Ilha do Cardoso (PEIC), Parque Estadual Pico do Marumbi (PEPM), Parque Estadual Turístico do Alto Ribeira (PETAR), Parque Nacional da Serra da Bocaina (PNSB), Parque Nacional da Serra dos Órgãos (PARNASO), and Reserva Biológica Augusto Ruschi (RBAR) (FIG. 1). Additional specimens from the states of Minas Gerais, Rio Grande do Sul, Rondônia, São Paulo, and Santa Catarina were obtained from the herbaria SP [Herbarium of the Instituto de Pesquisas Ambientais (IPA)] and FLOR [Fungarium of the Universidade Federal de Santa Catarina (UFSC)] and from two private individuals, with consent.

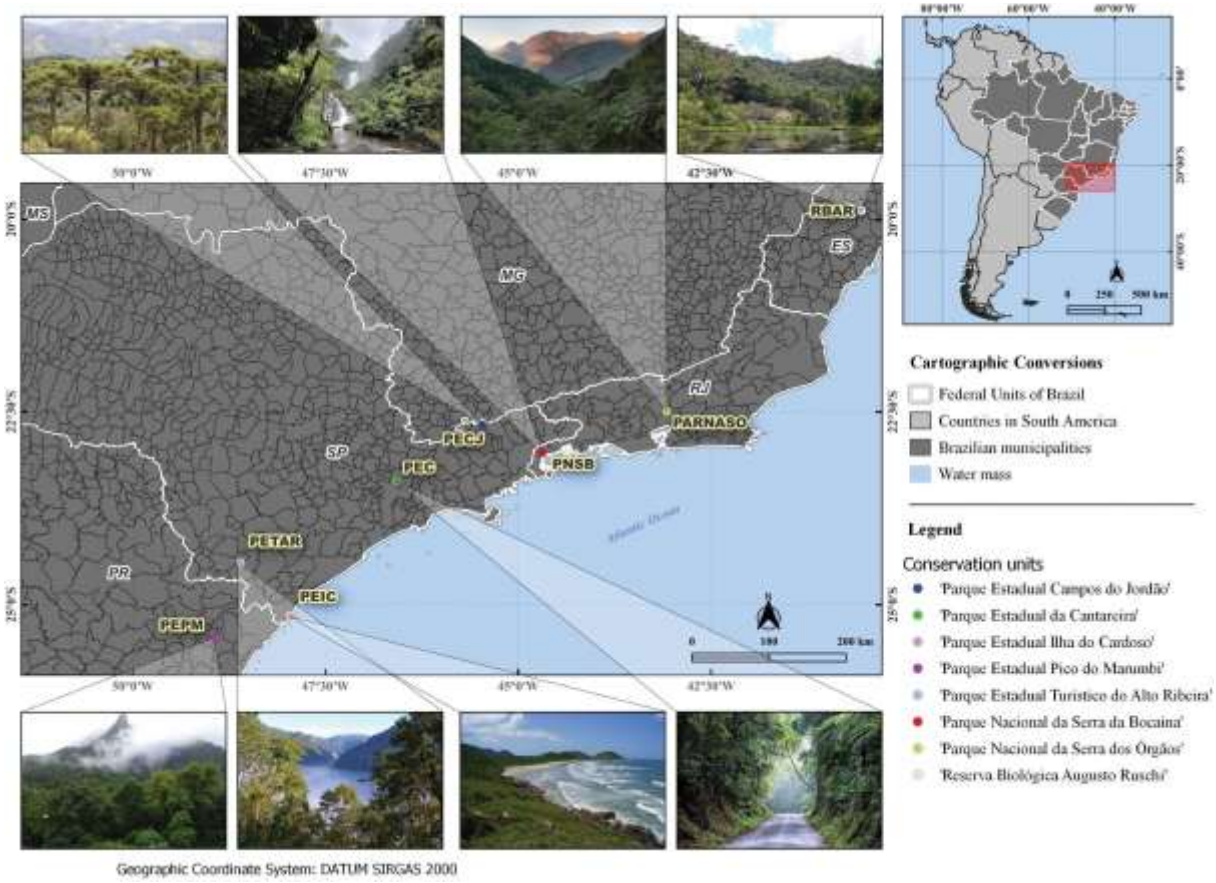

Figure 1. Geographic location of the sampling sites indicated as different color dots on the map with general vegetation aspects in the Brazilian states of Espírito Santo (ES), Rio de Janeiro (RJ), São Paulo (SP), and Paraná (PR).

The Brazilian Atlantic Rainforest hosts one of the world's most diverse tropical forest biota, supporting one of the highest degrees of species richness and rates of endemism on the planet (Mittermeier et al. 2004; Myers et al. 2000; Ribeiro et al. 2011). It is heterogeneous and composed of many different forest formations (dense ombrophilous, open ombrophilous, mixed ombrophilous, semideciduous seasonal, and deciduous seasonal) and a diversity of climates (tropical humid, subtropical of altitude, subtropical dry winter, and temperate) (Ab'Sáber 2003; Joly et al. 2014), with the annual average temperature ranging from 11.3 to 27.9 C, annual precipitation ranging from 643 to 3525 mm, and elevations from sea level to 2700 m above sea level (a.s.l.) (Joly et al. 2014; Oliveira-Filho 2017).

Data from the Global Biodiversity Information Facility (GBIF), which covers data from iNaturalist (https://www.inaturalist.org.https://mushroomobserver.org), were consulted to access the geographic occurrence of *Pseudohydnum* specimens from the American continent. Photos from the aforementioned platforms were downloaded to illustrate the macromorphological diversity of putatively undescribed *Pseudohydnum* species. We followed the Creative Commons (https://creativecommons.org/) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CCBY, CCBYNC, CCBYSA, CCBYND, CCBYNCND, and CCBYNCSA; for licenses and code attributions, see: https://creativecommons.org/licenses/?lang=en).

This study is in accordance with the Brazilian legislation on access to biodiversity and is registered in the "Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado" (SisGen no. A6F2384).

Morphological methods, notation, and use of standards.—To assess the macromorphological features, basidiomata images were taken using a Canon EOS 6D Mark II camera (Canon, Tokyo, Japan) fitted with a 100 mm macro lens, and the habitat, altitude, substrate, and nearby vegetation were documented. Basidiomata were dried in a hot air dehydrator (45 C) or in allochroic silica gel for their preservation. Color notation of fresh basidiomata is according to Kornerup and Wanscher (1978), with color codes noted in parentheses (e.g., 6E8, 7E5–6). Details of the basidioma surface were examined under a Zeiss Stemi 305 stereo microscope (Carl Zeiss, Oberkochen, Germany).

Microscopic analyses were performed from sections of fresh or rehydrated tissues under a Zeiss Axioscope 5 microscope (Carl Zeiss) with bright-field, phase-contrast optics, and a charge-coupled device camera (Zeiss Axiocam 208 color; Carl Zeiss). Dried samples were

rehydrated in distilled water or moistened in ethanol 70%, and microsections were cut by hand with a razor blade. Microscopic structures were examined in water, 3% KOH, 1% phloxine B ($C_{20}H_4Br_4Cl_2K_2O_5$), cotton blue, and Melzer's reagent, separately. At the beginning of a set of basidiospore data, the abbreviation [a/b/c] signifies "a" basidiospores measured from "b" basidiomata of "c" collections. Dimensions of basidiospores are presented in the following form: (m–)n–o(–p), in which "m" is the smallest value observed or calculated and "p" is the largest value observed or calculated. In the range of values observed or calculated, the 5th percentile is "n" and the 95th percentile is "o." Biometric variables are defined as follows:

L_m (W_m) = the average of all lengths (widths) of basidiospores for each collection, presented as a range of values.

Q = the ratio of length to width of a basidiospore or the range of such ratios for all basidiospores measured.

Q_m = the average of all Q values computed for each collection, presented as a range of values. The form of the basidiospores was interpreted based on the Q values, following Bas (1969), and descriptive terms for other morphological features follow Vellinga and Noordeloos (2001) and Spirin et al. (2023). Morphological characters from previously described *Pseudohydnum* species included in the dichotomous key are according to Zhou et al. (2022).

The width of the basidia was taken at the widest part, and the length was measured from the apex without sterigmata to the basal septum.

The collections examined including the selected nomenclatural types are deposited at the Fungarium IFungiLab (FIFUNGI) from the Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), and some duplicates were deposited at the herbarium SP. The herbaria abbreviations follow Thiers (2024, continuously updated).

DNA extraction, PCR amplification, and sequencing.— Genomic DNA was extracted from basidiomata dried in silica gel or from herbarium specimens, according to the instructions of the DNeasy Plant Mini Kit (Qiagen, San Diego, California) or the GenElute Plant Genomic DNA Miniprep Kit (MilliporeSigma, Burlington, Massachusetts).

The polymerase chain reactions (PCRs) were conducted on a Mastercycler Nexus GX2 thermal cycler (Eppendorf, Hamburg, Germany). The following primers were used for amplification and sequencing: ITS1F/ITS4, ITS1/ITS4B, or ITS8F/ITS6R (Dentinger et al. 2010; Gardes and Bruns 1993; White et al. 1990) for the nuc rDNA internal transcribed spacer region (ITS: ITS1-5.8S-ITS2); LR0R/LR5 or LR0R/ LR7 (James et al. 2006; Vilgalys and Hester 1990) for part of the nuclear ribosomal large subunit (also known as nuc 28S rDNA gene, 28S or LSU); and gRPB1-Af/fRPB1-Cr (Matheny et al. 2002; Stiller and Hall 1997) for part of the RNA polymerase II largest subunit

(*RPB1*). The amplification reaction mixture contained 25 µL Taq Pol Green Master Mix (2×) (Cellco Biotech, São Carlos, Brazil), 2.5 µL of each primer, 15 µL of ultrapure water, and 5 µL of template DNA. The thermal profile of PCR for rDNA markers was according to Binder and Hibbett (2003). The amplification of the protein-coding gene *RPB1* employed a touchdown PCR protocol, as detailed by Justo and Hibbett (2011). After visualization of positive PCR products on a 2% agarose gel with SafeDye Nucleic Acid Stain (Cellco Biotech, São Carlos, Brazil), PCR products were cleaned up prior to sequencing using QIAquick PCR Purification Kit (Qiagen) or PCR Purification Kit DPK-106 (Cellco Biotech, São Carlos, Brazil) as per manufacturer's guidelines.

Sequencing (Sanger method) was carried out at the Centro de Estudos do Genoma Humano e Células-Tronco at the Universidade de São Paulo (CEGH-USP, São Paulo, Brazil) or at Macrogen Korea (Seoul, South Korea). Sequencing was carried out using the same primers as those used for PCR.

Alignment and molecular phylogenetic analyses.— Bidirectional sequencing results were assembled using Geneious Prime 2022.1.1 (Biomatters, Auckland, New Zealand). Low-quality nucleotide sites at both ends of the sequences were pruned. All new sequences from this study were deposited in GenBank (http://www.ncbi. nlm.nih.gov/nuccore/). Sampling for reference sequences of *Pseudohydnum* as well as for the phylogenetic backbone of *Auriculariales* was according to Lutzoni et al. (2004), Porter et al. (2008), Malysheva and Spirin (2017), Yuan et al. (2018), Liu et al. (2022), Li et al. (2022), Zhou et al. (2022), Zhou et al. (2023), and Spirin et al. (2023), or selected by using the BLASTn search function of the National Center for Biotechnology Information (NCBI) database to find similar matches with taxa in *Pseudohydnum*. The supplementary table gives all taxa, collection information, and GenBank numbers of sequences used in this study (SUPPLEMENT 1).

Three data sets were assembled for this study. Dataset I (ITS+28S), containing representatives of all *Pseudohydnum* species with molecular sequences available as well as the backbone species of Auriculariales, was used to evaluate the phylogenetic placement of *Pseudohydnum* species in an order-level scheme. This data set was concatenated and included sequences of *Bjerkandera adusta* (Willd.) P. Karst. and *Sistotrema brinkmannii* (Bres.) J. Erikss. as outgroup (Li et al. 2022). Data set II (ITS+28S) encompasses virtually all available sequences of *Pseudohydnum* species (very short and/or incongruent sequences were excluded) and sequences of *Protomerulius substuppeus* (Berk. & Cooke) Ryvarden and *Protomerulius subreflexus* (Lloyd) O. Miettinen & Ryvarden as outgroup. This second data set was employed to gather only *Pseudohydnum* sequences for a more optimized alignment by preventing the loss of information due to the high variability in the ITS region and misaligned blocks in ITS matrix

when sequences of highly diverse species are included in a single data set (Oliveira et al. 2020). Data set III (ITS+28S+*RPB1*) comprises all *Pseudohydnum* representatives with *RPB1* sequences available in GenBank, thereby representing the vast majority of the genus' lineages in an outgroup-free (midpoint rooting) scheme.

Alignments for individual gene regions were made using MAFFT 7490 as implemented in Geneious (Katoh et al. 2002; Katoh and Standley 2013), adjusting the direction of nucleotide sequences according to the first sequence and selecting the E-INS-i iterative refinement method. Alignments were manually adjusted with AliView 1.17.1 (Larsson 2014) to minimize ambiguously aligned sites. Individual marker alignments were concatenated using Mesquite 3.70 (Maddison and Maddison 2023). The conflicts between the three genes under consideration were tested using PAUP* 4.0b10 (Swofford 2002). The results of the phylogenetic signals in the three genes were not in conflict. The concatenated data set comprising two loci was divided into four data partitions: ITS1, 5.8S, ITS2, and 28S, whereas the dataset comprising three loci was divided into three partitions: ITS, 28S, and *RPB1*. The combined alignments produced in this study can be found in TreeBASE (http://www.treebase.org/treebase-web/home.html; submission ID 31094) and as NEXUS file in Supplementary Material (SUPPLEMENT 2).

The phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian inference (BI) in the CIPRES Scientific Gateway (https://www.phylo.org/por tal2/home.action) (Miller et al. 2010). Maximum likelihood bootstrap analysis for phylogeny and assessment of branch support by bootstrap percentages (% BS) was carried out using RAxML-HPC 8 on XSEDE (8.2.12) (Stamatakis 2014) under the GTR+GAMMA model with 1000 bootstrap iterations. Bayesian analysis for the reporting of Bayesian posterior probability (BPP) support for branches was performed using MrBayes 3.2.7 on XSEDE (3.2.7a) (Ronquist et al. 2012). The best-fit nucleotide substitution model for each marker partition was determined with ModelTest 2 2.1.6, based on the Bayesian information criterion (BIC) (Posada and Crandall 1998). Four simultaneous Markov chains were run for 10 000 000 generations, and trees were sampled every 1000th generation (resulting in 10 000 trees). The first 25% trees, which represented the burn-in phase of the analysis, were discarded. The BPPs in the majority rule consensus tree were calculated by the remaining trees.

Phylogenetic trees from the ML and BI analyses were visualized with FigTree 1.4.4 (Rambaut 2018) and edited in CorelDRAW 2023 (Alludo, Ottawa, Canada) and Adobe Photoshop CC 2023 (Adobe Systems, São José, California). Branches that received BS and BPPs greater than or equal to 70% and 0.95, respectively, were considered significantly supported.

RESULTS

Phylogenetic relationships.—For data set I, the final alignment consisted of 196 sequences, comprising 37 newly generated sequences (18 ITS and 19 28S) and 159 sequences (83 ITS and 76 28S) downloaded from GenBank. The final alignment contained 1888 nucleotide sites including gaps (311 sites for ITS1, 176 sites for 5.8S, 319 sites for ITS2, and 1079 sites for 28S), of which 920 were conserved, 703 were parsimony-informative, and 265 were variable but parsimony-uninformative. For BI, the selected models for each DNA region of the concatenated data set were as follows: TPM2uf+G for ITS1 and ITS2, TPM2+I+G for 5.8S, and TIM1ef+I+G for 28S. The BI and ML analyses resulted in similar topologies, except the clade formed by *P. umbrosum*, which changed to be basal to the /*gelatinosum* clade in the ML tree instead of forming a single-species lineage in a polytomy at the /*gelatinosum* clade root in the BI tree. However, basal status of *P. umbrosum* in ML analysis does not get statistical support; thus, only the BI topology is presented as a master tree (FIG. 2) with BPP (≥0.95, first) and BS (≥70%, second) values labeled along the branches. In the boundaries delineated (FIG. 2), the genus *Pseudohydnum* forms a well-supported lineage (BPP = 0.95, BS = 92%) in Auriculariales, which splits into two major and well-supported clades referred to here as clade /*gelatinosum* (BPP = 1, BS = 93%) and clade /*viridimontanum* (BPP = 0.99, BS = 100%). The former encompasses 16 previously known species as well as three species new to science, formally introduced below (see Taxonomy) as *P. brasiliense, P. brunneovelutinum*, and *P. cupulisnymphae*. Meanwhile, the latter clade is a single-species lineage represented by a new taxon described herein as *P. viridimontanum*. This unexpected placement of the /*viridimontanum* clade at first gave rise to a proposal to split *Pseudohydnum* into two subgenera or even consider the *P. viridimontanum* lineage to be a putative distinct genus, but this phylogenetic placement was not corroborated based on the hypothesis inferred from data sets II and III. Furthermore, a thorough morphological analysis of *P. viridimontanum* collections has failed to identify any macro- and microanatomical differences that could justify any segregation from *Pseudohydnum*.

Despite some differences in branch relationships, analyses of the data set II, including virtually all available sequences of *Pseudohydnum*, resulted in the same lineages resolved in the topologies from data set I. The final alignment contained 2058 nucleotide sites including gaps (262 sites for ITS1, 170 sites for 5.8S, 277 sites for ITS2, and 1349 sites for 28S), of which 1545 were conserved, 391 were parsimony-informative, and 122 were variable but parsimony-uninformative. Substitution models were as follows: GTR+G (ITS1), TrNef (5.8S), TPM3uf+G (ITS2), and TIM1+I+G (28S). Phylogenetic trees obtained from ML and BI

analyses were similar in overall topologies and were not significantly different. The BI tree is shown in FIG.3. Sixteen previous *Pseudohydnum* species plus the four new taxa proposed here and some unidentified or misidentified collections were recovered in a fully supported clade (BPP = 1, BS = 100%). Eight main lineages (subclades) with high support could be recognized in the ingroup. Of them, seven overlap with those recovered in the recent ITS+28S–based phylogeny of Spirin et al. (2023) and one constitutes a new lineage represented by *P. brasiliense, P. brunneovelutinum*, and *P. cupulisnymphae. Pseudohydnum viridimontanum* is nested within lineage 1 (BPP = 0.99), alongside *P. umbrosum* from East Asia. This phylogenetic placement was not congruent with the topology retrieved from data set I, which resolves *P. umbrosum* as single-species lineage in the /*gelatinosum* clade, whereas *P. viridimontanum* lay in a distinct lineage (/*viridimontanum*) basal to the *Pseudohydnum* core clade.

Among all the resolved lineages, the second and third (FIG. 3) demand a more careful taxonomic interpretation, which is corroborated by Spirin et al. (2023) in a study that regarded both lineages as species complexes. In our analyses, lineage 2 is represented by *P. translucens* (= *P. candidissimum*) from East Asia, *P. himalayanum* and *P. sinobisporum* from China, *P. alienum* from Europe, and *P. omnipavum* from the northwestern part of North America. In addition, two sequences identified as "*P. gelatinosum*" from Canada (MN992495 and MN992496) and two highly similar sequences of *Pseudohydnum* sp. from Mexico (KC152166 and KT875091) were recovered in this lineage. As reconstructed (FIG. 3), three main subclades can be distinguished within lineage 2. The first encompasses *P. sinobisporum*, which formed a significantly supported clade (BS = 77%) in the ML tree, appearing as sister to the Mexican *Pseudohydnum* sp. sequences, with strong support (BPP = 0.99, BS = 97%). The second consists of a cluster of sequences belonging to species from the temperate-boreal forests of the Northern Hemisphere, viz., *P. himalayanum*, "*P. gelatinosum*," *P. translucens*, and *P. alienum*. These species nest in a moderately supported clade with *P. alienum* assuming a basal position to the "*P. himalayanum* + "*P. gelatinosum*" + *P. translucens*" branch; all independent clades were well supported (BPP = 1, BS = 100%; BPP = 1, BS = 100%; BS = 76%; BPP = 0.99, BS = 76%; respectively). Finally, in the third subclade, *P. omnipavum* appears as a single-species lineage with full support (BPP = 1, BS = 100%). In lineage 2, "*P. gelatinosum*" (QFB28581, QFB28623) from Canada and *Pseudohydnum* sp. (CB 08107, GO-2009-433) from Mexico clearly represent undescribed species and, thus, require proper morphological studies to support their recognition.

Figure 2. Bayesian inference (BI) tree of *Pseudohydnum* in Auriculariales inferred from the concatenated data set of ITS and 28S regions. GenBank accession numbers and country of origin follow taxon name. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BPP and 1.0 BS support. The tree is rooted to *Bjerkandera adusta* (HHB-12826-Sp) and *Sistotrema brinkmannii* (isolate 236). The new species are presented in bold under color-highlighted clades, with an indicative legend in the upper left and illustration of representative specimen in the right. Black stars represent holotype specimens.

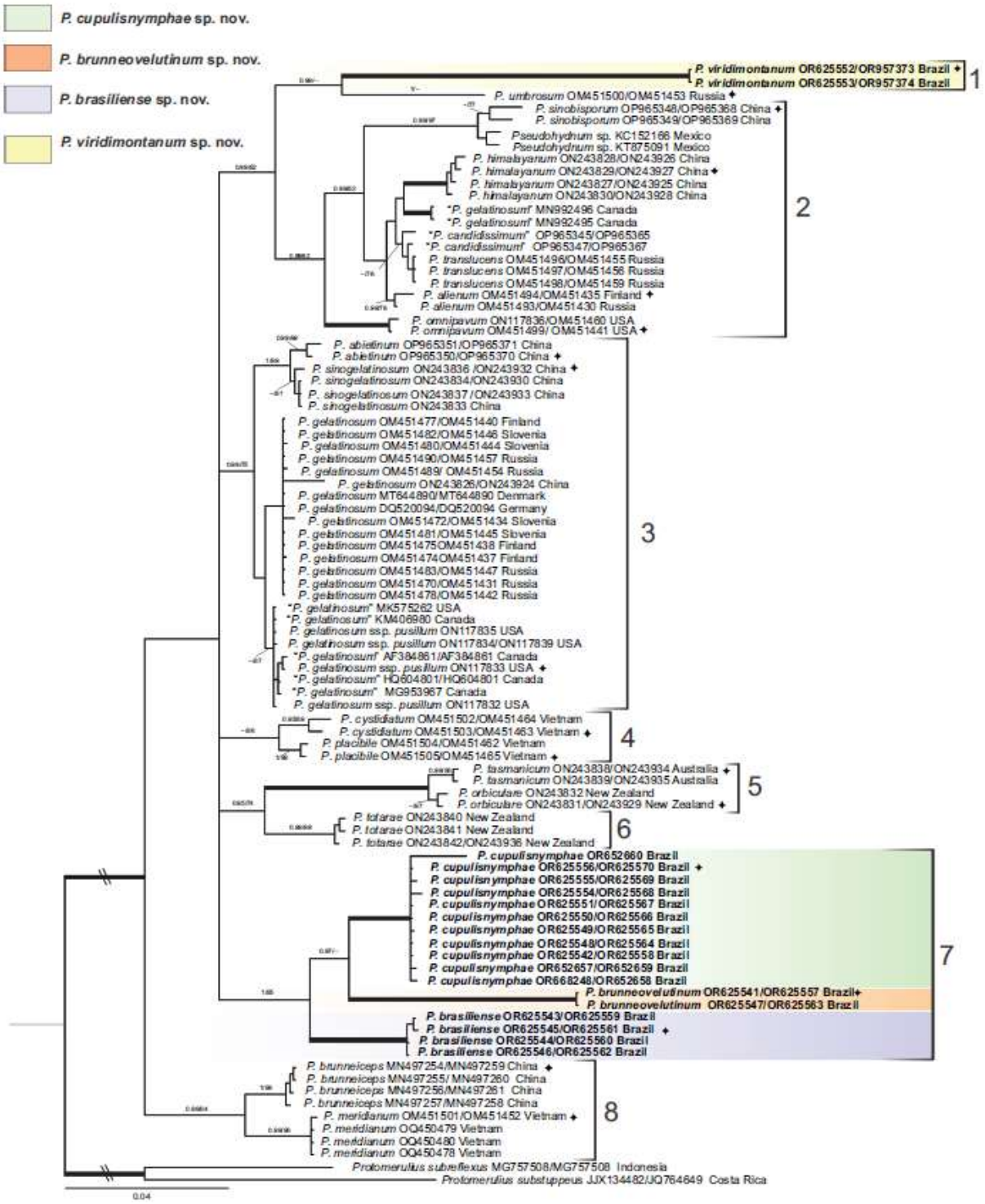

Figure 3. Combined phylogenetic ITS+28S topology from Bayesian analysis for *Pseudohydnum* spp. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BS and 1.00 BPP support. The eight main lineages inferred are enumerated. The tree is rooted to *Protomerulius subreflexus* (OM 14402.1) and *P. substuppeus* (O 19171). The new species are presented in bold under color-highlighted clades, with an indicative legend in the upper left and illustration of representative specimen in the right. Black stars represent holotype specimens.

In lineage 3, Eurasian sequences of *P. gelatinosum* s.s. grouped within a well-defined but not strongly supported subclade, sister to the North American *P. gelatinosum* ssp. *pusillum*. The latter is grouped within a well-supported subclade (BS = 87%) in the ML tree, which also includes a number of sequences generically identified as "*P. gelatinosum*" from North America. This phylogenetic placement is consistent with that of the data set I tree, in which the *P. gelatinosum* ssp. *pusillum* clade was strongly supported (BPP = 0.96, BS = 94%). *Pseudohydnum abietinum* and *P. sinogelatinosum* also cluster in the third lineage, appearing as sisters to the branch bearing "*P. gelatinosum* s.s. + *P. gelatinosum* ssp. *pusillum*," with significant support (BPP = 0.99, BS = 75%).

Finally, for data set III (ITS+28S+*RPB1*), the final alignment consisted of 87 sequences, including six newly generated *RPB1* sequences. The final alignment contained 2631 nucleotide sites (604 sites for ITS, 894 sites for 28S, and 1133 for *RPB1*), of which 2160 were conserved, 415 were parsimony-informative, and 56 were variable but parsimony-uninformative. Substitution models were as follows: TIM2ef+G (ITS1), GTR+I+G (LSU), and TrN+I (*RPB1*). The overall topologies generated through ML and BI analyses were found to be highly consistent, with no significant differences. The BI tree is shown in FIG. 4. The phylogenetic inference from this data set fully supports all the new introduced species, and it is in accordance with the topology derived from data set II.

TAXONOMY

Based on our morphological, ecological, and molecular phylogenetic data, four new species of *Pseudohydnum* from Brazil are described and illustrated here.

Pseudohydnum brasiliense C. Coelho-Nascimento, D.A. Zabin & Menolli, sp. nov.
MycoBank MB851356

Typification: BRAZIL. SÃO PAULO: Campos do Jordão, Parque Estadual Campos do Jordão, Trilha do Rio Sapucaí, from decaying angiosperm wood, 21 Jan 2020, *M.P. Drewinski, N. Menolli Jr., M.F.O. Silva, M.P.C. Santos & T.R. Santos MPD570* (**holotype** FIFUNGI279), GenBank: ITS = OR625545, 28S = OR625561, *RPB1* = PP805851.

Diagnosis: Differs from other known *Pseudohydnum* species in having basidiomata with a lateral well-developed stipe, yellowish gray to grayish brown or fawn brown pileus surface, hymenophoral spines 2.5–4 per mm, simple hyphidia, and subglobose to broadly ellipsoid basidiospores.

Etymology: brasiliense (Latin) refers to the country of the type locality.

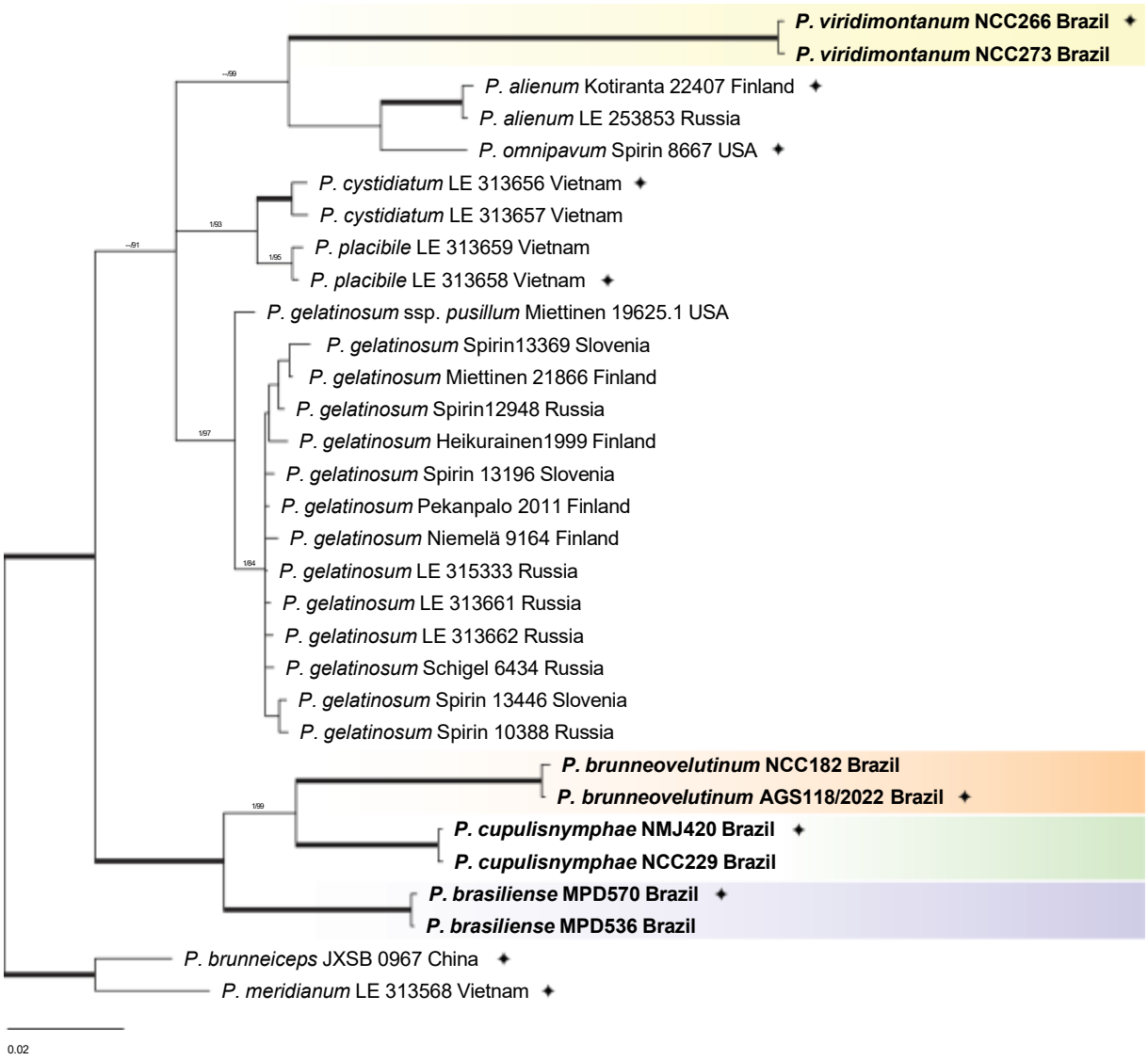

Figure 4. Combined phylogenetic ITS+28S+RPB1 topology from Bayesian analysis for *Pseudohydnum* spp. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BS and 1.00 BPP support. The tree is midpoint-rooted. The new species are presented in bold under color-highlighted clades. Black stars represent holotype specimens.

Morphology: Basidiomata small- to medium-sized, gelatinous when fresh, pileate, stipitate, solitary or gregarious (not confluent at the base), irregularly subglobose in young stages, becoming more or less trumpet-shaped in profile before the complete pileus expansion. Pileus up to 42 mm, spathulate to flabellate or pseudoinfundibuliform, surface minutely velutinous to pubescent, yellowish gray (4B2) to grayish brown (6D3, 6EC) or fawn brown (7E4), translucent when wet; margin sharp, sometimes undulating. Hymenophore white, hydnoid. Hymenophore spines sharp-tipped, 0.4–2.5 mm long, 2.5–4 per mm, white (1A1) to yellowish white (1A2). Stipe 6–11 × 3.5– 6 mm, white (1A1) to yellowish white (1A2) or yellowish gray (4B2), translucent; surface covered by short spines to minutely grandinioid or almost smooth. Context translucent, unchanging. Odor mild. Taste mild or pleasant.

Hyphal system monomitic, generative hyphae with abundant clamp connections, hyphae unchanged in KOH. Pileal surface trichodermial to subtrichodermial, fascicles abundant of sinuate to slightly sinuate hyphae (3.0–10.0 µm diam) that are colorless or pale grayish brown in mass and frequently septate, thin- to slightly thick-walled; terminal elements poorly differentiated. Contextual hyphae of two types: (i) dominant thin- to slightly thick-walled, smooth, hyaline, interwoven, commonly branching, frequently anastomosing, 1.0–3.0 µm diam; and (ii) occasional thin-walled, slightly inflated up to 15.0 µm diam. Spine tramal hyphae thin-walled, smooth, hyaline, moderately branched, interwoven, 1.5–3.0 µm diam. Hyphidia covering basidial cells, thin-walled, smooth, sometimes slightly flexuous, simple, slightly swollen toward the apex; apex cylindrical to subfusiform, usually slightly constricted, 1.6–2.5 µm diam; hyphidia and basidia derived from the same hyphae. Basidia mostly 2-celled (bisporic), rarely 4-celled, longitudinally septate, multigutullate, barrel-shaped, ellipsoid to subglobose, 9.0–11.3 × 7.0–8.6 µm; sterigmata 4.5–15.2 × 1.6–3.5 µm, swollen or sometimes aciculate, occasionally constricted. Probasidia mostly subglobose to globose or lageniform, 8.0–8.7 × 5.9–7.0 µm, usually multigutullate. Basidiospores [240/8/8] (5.7–)6.4–9.7(–10.5) × (4.7–)5.4–8.8(–9.5) µm [L_m = 7.3–7.9 µm; W_m = 6.2–6.8 µm; Q = (1.02–)1.06–1.32(–1.39); Q_m = 1.16–1.18], subglobose to broadly ellipsoid, rarely globose or ellipsoid, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, often germinating by repetition or by germ tubes on the surface.

Habitat and distribution: Growing erect and ascendant on decayed angiosperm wood in dense and mixed ombrophilous forest fragments of Southeastern Brazil. Also found on *Pinus taeda* L. wood in a seasonal semi-deciduous forest of Southern Brazil. Known for the Brazilian states of São Paulo, Rio de Janeiro, Rondônia, and Santa Catarina.

Other specimens examined: BRAZIL. RIO DE JANEIRO: Teresópolis, Parque Nacional da Serra dos Órgãos, Trilha da Pedra do Sino, from decaying angiosperm wood, 14 Dec 2019, *M.P. Drewinski, J.A.D. Barbosa, T.S. Cabral & C.S. Carvalho MPD536* (FIFUNGI281), GenBank: ITS = OR625543, 28S = OR625559, *RPB1* = PP805850; ibid., 16 Dec 2019, *M.P. Drewinski, J.A.D. Barbosa, T.S. Cabral & C.S. Carvalho MPD562* (FIFUNGI282), GenBank: ITS = OR625544, 28S = OR625560; SÃO PAULO: Campos do Jordão, Parque Estadual Campos do Jordão, 27 Jan 1987, *D.N. Pegler, K. Hjortstam & L. Ryvarden KH 3858* (SP214396); ibid., 28 Jan 1987, *D.N. Pegler, K. Hjortstam & L. Ryvarden KH 3859* (SP214385); Trilha do Rio Sapucaí, from decaying *Pinus taeda* wood, 21 Jan 2020, *M.P. Drewinski, N. Menolli Jr., M.F.O. Silva, M.P.C. Santos & T.R. Santos MPD572* (FIFUNGI280), GenBank: ITS = OR625546, 28S = OR625562; Cananeia, Ilha do Cardoso, 3 Feb 1987, *M. Capelari, R. Maziero & M.I.S.M.C. Castro 1241* (SP211805); RONDÔNIA: Jarú, Reserva Biológica do

Jarú, on the right bank of the Ji-Paraná River, from decaying angiosperm wood, 13 May 1987, *M. Capelari, R. Maziero & M.R.O. Santos MC1456* (SP212177); SANTA CATARINA: Urubici, Reserva Particular do Patrimônio Natural (RPPN) Portal das Nascentes, Trilha da Água, from decaying angiosperm wood, 9 Jan 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, E.L. Gumboski, E.S. Alves, & P.R. Pezzuto MIND.Funga0397* (FLOR 71400), GenBank: ITS = PP790410.

Notes: The presence of a well-developed stipe differentiates *P. brasiliense* from other known species of *Pseudohydnum* and those described in the present paper. It also has a distinct pileus color variation, ranging from yellowish gray (collections MPD570 and MPD572) to fawn brown (collection MPD536), with occasional pale brown to watery-gray tinges in rain washed basidiomata (collections MPD562 and MIND. Funga0397). The fawn brown pileus of *P. brasiliense* in the collections MPD536 and MIND.Funga0397 evoke *P. brunneovelutinum* in the field. However, the latter can be distinguished by a number of characters, including the short to rudimentary stipe, presence of dendrohyphidia, and smaller basidiospores (5.9–6.8 × 5.2–6.3 µm). *Pseudohydnum brasiliense* can also be compared with *P. brunneiceps* because of their similar large basidiospores (6–8.9 × 5.5–7.1 µm, Q_m = 1.11–1.17 in *P. brunneiceps*) and well-developed stipe, but the latter has larger basidiomata with a maximum reported pileus diameter of 80 mm, the hyphidia are richly branched, and its ecology and distribution are different because it is so far known only from the southern and southeastern subtropical zone of China, where it inhabits decayed wood of conifers, mainly *Cryptomeria japonica* (Thunb. ex L. f.) D. Don (Chen et al. 2020; Spirin et al. 2023).

Pseudohydnum brasiliense also deviates from its closest relatives, except *P. brunneiceps*, in the presence of mostly bisporic basidia, unique among the species introduced herein. A very similar collection with remarkable bisporic basidia was described from French Guyana as *P. gelatinosum* var. *bisporum* (Courtecuisse and Lowy 1990). From an anatomical perspective, the latter closely resembles *P. brasiliense* because, in addition to bisporic basidia, both share similar pileal surface hyphae, width of contextual/tramal hyphae, and basidiospores. Conversely, neither macromorphological descriptions nor basidioma line drawings were provided in Courtecuisse and Lowy's protolog. These authors ascribed the same macromorphological concept of the type variety presented by Lowy (1971) for its new varietal name, which includes sessile to short-stipitate basidiomata. This latter trait deviates from the well-developed stipe delivered by *P. brasiliense*. Thus, despite the somewhat divergent macromorphology, we interpret that *P. gelatinosum* var. *bisporum* is likely conspecific with *P. brasiliense*, and the diagnostic well-developed stipe was an overlooked trait in the former's

protolog. However, we cautiously refrain from proposing a synonymy between them until the authentic material is fully reexamined to support our viewpoint.

Bastos et al. (2015) identified a collection from the Brazilian Amazon Forest as *P. gelatinosum* based on the description provided by Lowy (1971). However, judging from descriptions and pictures, the specimen from the Amazon Forest barely resembles Lowy's material, from which it differs by the shorter basidiospores (6–7 × 8– 5 μm) and the presence of a well-developed stipe (Bastos et al. 2015). With the exception of larger basidiospores, the collections of *P. brasiliense* herein examined mostly agree to that studied by Bastos et al. (2015), especially by having similar pileus dimensions and a well-developed stipe; thus, they may be conspecific, although a careful reexamination of the samples studied by them is necessary in order to confirm this. A second Amazon collection, designated as "*P. gelatinosum*" and revised from herbarium SP (SP212177), is consistent with the *P. brasiliense* morphoanatomical concept, exhibiting a distinctly stipitate basidiomata, bisporic basidia, and subglobose to broadly ellipsoid basidiospores (6.3–8.3 × 5.3–7.2 μm, Q_m = 1.16). Unfortunately, sequences from this old collection could not be obtained to support the morphoanatomical evidence.

In the phylograms depicted (FIGS. 2 and 3), *P. brasiliense* is sister to the "*P. brunneovelutinum* + *P. cupulisnymphae*" clade, with which all together forming a well-supported lineage (BPP = 1, BS = 85%) of Neotropical species. Nevertheless, no shared macro- and microanatomical traits were identified between these three species that could support their clustered phylogenetic placement, except the basidiospores commonly germinating by repetition. However, this latter feature should not be overemphasized in taxonomic terms, since it seems to be a common trait present in a number of *Pseudohydnum* species. Differences of *P. brasiliense* from the phylogenetically close *P. cupulisnymphae* are discussed under this species.

Pseudohydnum brunneovelutinum C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 8, 9

MycoBank MB851357

Typification: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, from decaying angiosperm wood, 19 Nov 2022, *A.G.S Silva-Filho & C.A.V. Fraga Jr. AGS118/2022* (**holotype** FIFUNGI283), GenBank: ITS = OR625541, 28S = OR625557, *RPB1* = PP805852.

Diagnosis: Differs from other known *Pseudohydnum* species in having flabelliform to petaloid basidiomata with a short to rudimentary stipe base, cocoa brown to brown or dark

brown pileus surface, sparse spines up to 1.5 per mm at base, conspicuous dendrohyphidia, and globose to broadly ellipsoid basidiospores.

Etymology: brunneo (Latin) = brown; *velutinum* (Latin) = velvety, densely covered with fine, short, soft, erect hairs; referring to the brown velutinous pileal surface of the specimens.

Morphology: Basidiomata small-sized, gelatinous when fresh, growing solitary or in pairs (then basally coalescent), at first as a protruding jelly tongue with an incurved edge, then flabelliform to petaloid with a short stipe. Pileus up to 35 mm in widest dimension, surface initially smooth, then velutinous to pubescent, cocoa brown (6E6) to brown (6E8, 7E5–6) or dark brown (5F6–8, 6F6–8), not translucent; margin sharp, even, fertile. Hymenophore white, hydnoid. Hymenophore spines sharp-tipped, white (1A1), up to 1.5 per mm, of two types: (i) longer well-developed spines, 1–2 mm long; and (ii) very small spines up to 0.3 mm long. Stipe short, up to 15 mm long, sometimes rudimentary, concolor with pileal surface; surface covered by spines or minutely papillate. Context translucent, pale brownish gray (9C2) to pale grayish brown (9D3), unchanging. Odor and taste mild.

Hyphal system monomitic, generative hyphae with abundant clamp connections, hyphae unchanged in KOH. Pileal surface as a compact trichoderm, with abundant fascicles of interwoven and vertically projected hyphae; hyphae 2.5–5 µm diam, thin- to slightly thick-walled, sometimes sinuous to tortuous, sparingly branched. Contextual hyphae thin- to slightly thick- walled, hyaline, colorless to pale yellow, frequently branching, straight to tortuous, interwoven, 1.8–3.5 diam. Spine tramal hyphae thin-walled, commonly branching, interwoven, 1.5–2.8 µm diam. Hyphidia conspicuous, strongly and irregularly branched (dendrohyphidial), thin-walled, slightly thick-walled toward the base, smooth, hyaline, partially covering basidia but sometimes projecting above hymenial layer up to 30 µm. Basidia mostly 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose or globose, 10.1–12.8 × 7.2–10.4 µm; sterigmata up to 20.6 × 2.2 µm, conic to aciculate or distinctly longer and tubiform with an obtuse apex. Probasidia subglobose to pyriform or short clavate, 9.0–10.5 × 4.7–8.4 µm, mostly multigutullate. Basidiospores [120/4/2] (5.3–)5.9–6.8(–7.7) × (4.6–)5.2–6.3(–6.7) µm [L_m = 6.3–6.5 µm; W_m = 5.7–5.8 µm; Q = (1.00–)1.02–1.38 (–1.51); $\mathbf{Q_m}$ = 1.11–1.15], globose to broadly ellipsoid, infrequently ellipsoid, inamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, occasionally germinating by repetition.

Habitat and distribution: Growing on unknown decayed angiosperm wood in dense ombrophilous forest fragments of Southeastern Brazil. Known for the Brazilian states of Espírito Santo and São Paulo.

Other specimen examined: BRAZIL. SÃO PAULO: São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, Trilha das Figueira, from decaying angiosperm wood, 4 Feb

2022, *C. Coelho-Nascimento NCC182* (FIFUNGI284), GenBank: ITS = OR625547, 28S = OR625563, *RPB1* = PP805853.

Notes: Pseudohydnum brunneovelutinum deviates from all other known *Pseudohydnum* species due to the presence of conspicuous dendrohyphidia, which is unique within the genus. Thus, it can hardly be mistaken by microscopic examination, even in the absence of molecular information. From a macromorphological point of view, *P. brunneovelutinum* may evoke *P. orbiculare* in the field because of the dark brown and velutinous pileus surface and the hymenophore with sparse spines, but the latter, described from New Zealand's South Island, has sessile basidiomata and larger basidiospores, 6.5–7.9 × 5.6–6.8 µm (Zhou et al. 2022). Because of their similar pileus surface color, *P. brunneiceps, P. meridianum*, and *P. umbrosum* can also be compared with *P. brunneovelutinum*. However, *P. brunneiceps* and *P. umbrosum*, both species poorly sampled from the Asian continent (Chen et al. 2020; Spirin et al. 2023), differ from *P. brunneovelutinum* by the denser hymenophoral spines and much larger basidiospores, 7.6–9.8 × 6–7.1 µm for *P. brunneiceps* and 6.9–8.9 × 6.1–7.1 µm for *P. umbrosum* (Chen et al. 2020; Spirin et al. 2023), whereas *P. meridianum*, presently known from Vietnam, can be distinguished from *P. brunneovelutinum* by a stouter habit, the pronounced vertical stipe, and the nearly smooth pileal surface, as well as slightly smaller basidiospores (5.1–6.2 × 4.9– 5.8 µm) with lower Q values (Q = 1.0–1.1) (Spirin et al. 2023).

Phylogenetically, *P. brunneovelutinum* was recovered as sister to *P. cupulisnymphae* with high support (BPP = 0.98, BS = 84%). Differences between these phylogenetically close species are discussed under *P. cupulisnymphae*.

Pseudohydnum cupulisnymphae C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 10, 11 MycoBank MB851358

Typification: BRAZIL. RIO DE JANEIRO: Paraty, trail to Sítio São José, Parque Nacional da Serra da Bocaina, from decaying angiosperm wood, 3 Dec 2021, *N. Menolli Jr., N. Rigo, A. Sandoval & J. Ferreira NMJ420* (**holotype** FIFUNGI285), GenBank: ITS = OR625556, 28S = OR625570, *RPB1* = PP805855.

Diagnosis: Differs from other known *Pseudohydnum* species in having mostly sessile basidiomata, orbicular to conchiform or irregularly lobed pileus, whitish to grayish yellow or pale pinkish orange pileus surface, hymenophoral spines 3.5–6 per mm, simple to sparingly branched hyphidia, and subglobose to broadly ellipsoid basidiospores (5.8–8.0 × 5.1–7.0 µm).

Etymology: cupulis (Latin) = cup-like shape; *nymphae* (Latin) = fairy; referring to the distinct cup shape of young basidiomata that resembles mystic vessels offered by fairy folk in "Fairy cup legends" (Migratory Legends of Christiansen Type 6045).

Morphology: Basidiomata small to somewhat large-sized, gelatinous when fresh, pileate, sessile to substipitate, solitary or in grouped tiers, sometimes basally coalescent and sharing an extended attachment base, at first forming a globose primordium with a short peduncle, then distinctly funnel-shaped or cupulate, and finally orbicular, flabelliform to flat conical or irregularly shaped. Pileus 13–40(–90) mm diam, surface initially covered by crowded tiny spines, becoming smooth to minutely velutinous or papillate, whitish to grayish yellow (1B3, 3B2) or pale pinkish orange (5A2, 6A3), translucent; margin blunt, even to crenate or irregularly lobed. Hymenophore white to yellowish white, hydnoid. Hymenophore spines sharp-tipped, 1–3 mm long, 3.5–6 per mm, white. Stipe absent or rudimentary. Context up to 15 mm thick, translucent, unchanging. Odor mild or sweet at first, then pleasantly fruity like apricots. Taste mild.

Hyphal system monomitic, composed of clamped generative hyphae, sometimes distinct clamps only in the hymenium, hyphae unchanged in KOH. Pileal surface subtrichodermial to trichodermial, composed of abundant fascicles of cylindrical hyphae (2.5–8.5 µm diam) that are colorless or pale yellowish in mass and frequently septate, thin- to slightly thick-walled; terminal elements narrowly clavate to conical or narrowly utriform. Contextual hyphae thin- to slightly thick-walled, hyaline, branching, interwoven, frequently anastomosing, some with incrustations, 2.0–3.6 µm diam. Spine tramal hyphae thin-walled, smooth, hyaline, commonly branched, interwoven, 1.0–4.2 µm diam. Hyphidia thin- to slightly thick-walled, smooth, rather constant in wide throughout their length, 1.0–3.0 µm diam at the apex, hyaline, spread among basidia and basidioles, mostly simple, sometimes sparingly branched, flexuous. Basidia strictly 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose or globose, sometimes broadly clavate, 10.0–14.0 × 8.5–11.0 µm; sterigmata up to 24 × 2.6 µm, tubiform, usually abruptly tapering at the apex. Probasidia subglobose to short clavate, 9.0–11.4 × 6.1–8.4 µm, mostly multigutullate. Basidiospores [420/14/14] (5.2–)5.8–8(–8.5) × (4.8–)5.1–7(–7.4) µm [L_m = 6.3–6.6 µm; W_m = 5.7–5.9 µm; Q = (1.00–)1.02–1.26(–1.28); Q_m = 1.10–1.18], subglobose to broadly ellipsoid, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, occasionally germinating by repetition.

Habitat and distribution: Common on rotten angiosperm wood in dense and mixed ombrophilous forest fragments of Southeastern and Southern Brazil; other recorded substrate includes the caudice of the tree fern *Dicksonia sellowiana* Hook. (“Samambaiaçu”). Known for the Brazilian states of Paraná, São Paulo, Santa Catarina, Rio de Janeiro, and Rio Grande do Sul, with putative records to Argentina, Bolivia, Guyana, Jamaica, Mexico, and Panama (Lowy 1959, 1971; Niveiro and Popoff 2011).

Other specimens examined: BRAZIL. PARANÁ: Morretes, Parque Estadual Pico do Marumbi, Trilha Antoninho Palmiteiro, from decaying angiosperm wood, 18 Jan 2023, *C. Coelho-Nascimento, A.G.S. Silva-Filho & M.D. Xavier NCC246* (FIFUNGI289), GenBank: ITS = OR625549, 28S = OR625565; ibid., 19 Jan 2023, *C. Coelho-Nascimento, A.G.S. Silva-Filho & M. D. Xavier NCC247* (FIFUNGI286), GenBank: ITS = OR625550, 28S = OR625566; RIO DE JANEIRO: Teresópolis, Parque Nacional da Serra dos Órgãos, Trilha Cartão Postal, from caudice of the tree fern *Dicksonia sellowiana*, 9 Dec 2022, *A.G.S. Silva-Filho, A.L.V. Ribeiro, M.E.C. Morais & C. Coelho-Nascimento NCC229* (FIFUNGI290), GenBank: ITS = OR625548, 28S = OR625564, *RPB1* = PP805854; RIO GRANDE DO SUL: Carambá do Sul, Parque Nacional dos Aparados da Serra, Trilha Fortaleza, from decaying angiosperm wood, 27 May 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, G. Alves-Silva & E.L. Gumboski MIND.Funga0893* (FLOR71600), GenBank: ITS = OR652657, 28S = OR652659; SANTA CATARINA: Benedito Novo, Pousada Campo do Zinco, from decaying angiosperm wood, 24 Nov 2020, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, G. Alves-Silva & E.L. Gumboski MIND.Funga0040* (FLOR71289), GenBank: ITS = OR668248, 28S = OR652658; Urubici, RPPN Portal das Nascentes, Trilha da Água, from decaying angiosperm wood, 9 Jan 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, E. Gumboski, E. dos Santos Alves, & P.R. Pezzuto MIND.Funga0348* (FLOR71400), GenBank: 28S = OR652660; Brusque, from decaying wood, 14 May 1964, *P. Reitz & K. Wells n/a* (SP71410); SÃO PAULO: São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, Trilha da Bica, from decaying angiosperm wood, 26 Feb 2021, *A.L.V. Ribeiro, M.P.C. Santos, A.C. Morais, C. Coelho-Nascimento, J.F. Amorim & A.M. Oliveira NMJ394* (FIFUNGI291), GenBank: ITS = OR625554, 28S = OR625568; Iporanga, Parque Estadual Turístico do Alto Ribeira, from decaying angiosperm wood, 4 Mar 2021, *N. Menolli Jr., M.P.C. Santos, D.A. Zabin, C. Coelho-Nascimento & N. Rigo NMJ398* (FIFUNGI294), GenBank: ITS = OR625555, 28S = OR625569; Cananeia, Parque Estadual da Ilha do Cardoso, Núcleo Perequê, Trilha Poço das Antas, from decaying angiosperm wood, 8 Jan 2019, *M.P. Drewinski MPD344* (FIFUNGI387), GenBank: ITS = OR625542, 28S = OR625558; Trilha da Cachoeira Grande, from decaying angiosperm wood, 1 Feb 2023, *C. Coelho-Nascimento, N. Menolli Jr. & A.G.S Silva-Filho NCC259* (FIFUNGI288), GenBank: ITS = OR625551, 28S = OR625567; Barra do Turvo, from decaying wood, 6 Dec 1973, *D.M. Vital n/a* (SP112455); São Vicente, Praia de Paranapuã, from decaying wood, 27 May 1993, *D. Matheus & L.K. Povineli CCB 432* (SP250989).

Notes: Pseudohydnum cupulisnymphae has been the most frequently encountered *Pseudohydnum* species in the sampled Atlantic Rainforest areas. It occurs from areas of dense ombrophilous forest in the state of Rio de Janeiro to areas of mixed ombrophilous forest in the state of Rio Grande do Sul. The funnel-shaped or cupulate young basidioma stages represent a diagnostic character that can differentiate *P. cupulisnymphae* from other species herein described. The other striking trait is the stout habitus and markedly lobed pileus margin displayed in the holotype collection (NMJ420; FIG. 11), although the basidioma size in the other studied collections did not exceed a maximum diameter of 40 mm.

Morphological segregation from taxa with overlapping geographic range requires comparisons of *P. cupulisnymphae* with *P. brasiliense* and *P. viridimontanum*. The first can be separated from *P. cupulisnymphae* by its stipitate basidiomata, which display a spathulate to pseudoinfundibuliform pileus, and larger basidiospores (6.4–9.7 × 5.4–8.8 μm) from mostly bisporic basidia. Differences from *P. viridimontanum* are discussed under this species.

For species without an overlapping geographic range, we limit the comparisons to only with other sessile Pseudohydnum species, such as *P. orbiculare*, *P. cystidiatum*, and *P. umbrosum*. Despite similar micromorphology, P. orbiculare is easily distinguished by its brownish pileus and very sparse hymenial spines (0.5–1 per mm) (Zhou et al. 2022). On the other hand, *P. cystidiatum*, although it shares with *P. cupulisnymphae* similar pileus color, produces distinctly smaller basidiomata (up to 1 cm diam) and larger basidiospores (6.8–9.5 × 5.6–7.6 μm) (Spirin et al. 2023). Lastly, *P. umbrosum* differs by having smaller basidiomata (up to 2 cm diam), a grayish brown pileus, and larger basidiospores (7.6–9.8 × 6–7.1 μm) (Spirin et al. 2023).

In arguments for novelty, we are also concerned with segregation/association of *P. cupulisnymphae* from previous Neotropical collections named as *P. gelatinosum*. Möller (1895) was the first to describe collections of *Pseudohydnum* [as *Tremellodon* (Pers.) Fr.] in Brazil from specimens found in Blumenau (Santa Catarina state). No authentic material was referenced, but illustrations are available (Möller 1895:tafel V, fig. 34). In Möller's description, a grayish, sessile, and stout specimen (up to 10 cm in diam) growing on rotten wood was identified as *Tremellodon gelatinosum* Pers. (= *Pseudohydnum gelatinosum*). He regarded *T. gelatinosum* as a common and widespread species and considered the Brazilian samples identical to the European lookalike. Judging from his description (Möller 1895), Möller's sessile samples named *T. gelatinosum* are clearly conspecific with *P. cupulisnymphae* because the two share similar habit, basidioma color and size, and basidiospore shape. However, *P. gelatinosum* s.s. from Europe exhibits a smaller pileus (not exceeding 40 mm), commonly stipitate basidiomata, and longer basidiospores (5.3–9 μm) (Spirin et al. 2023). Moreover, some collections of *P. gelatinosum* s.s display a grayish-brownish pileus surface

(Spirin et al. 2023), whereas brownish tinges were absent in all examined *P. cupulisnymphae* collections.

Descriptions of collections named as *P. gelatinosum* provided by Lowy (1959, 1971), based on collections from Bolivia, Brazil, Guyana, Jamaica, Mexico, and Panama, also immediately evoke *P. cupulisnymphae*, both macromorphologically and microanatomically, although broader basidiospore dimensions were reported for the collection described as *P. gelatinosum* var. *paucidentatum* (7–9 × 6–8.5 μm) when compared with *P. cupulisnymphae* basidiospore measurements. In addition to the atypical broader basidiospores, Lowy (1959) regarded the widely sparse spines as a distinctive trait to introduce the latter varietal name from a single collection from Bolivia. However, distinct sparse spines represent a common trait in some small-sized *Pseudohydnum* specimens, especially in species that exhibit a wide variation in the mature basidioma size, as observed for *P. cupulisnymphae* (FIG. 10G). Additionally, the size range of basidiospores should not be overemphasized as sole criterion for taxon segregation in *Pseudohydnum*, as it can exhibit a huge variation, as observed for *P. gelatinosum*, the most common representative of the genus in temperate-boreal forests of Eurasia (Spirin et al. 2023). Thus, based on a cautious interpretation of collections named "*P. gelatinosum*" by Lowy (1959, 1971), we formally consider their collections conspecific with *P. cupulisnymphae* and putatively consider *P. gelatinosum* var. *paucidentatum* to be its synonym, although a revision of the varietal collection type could confirm this.

Finally, we also ascribe the name *P. cupulisnymphae* to the collection reported as *P. gelatinosum* from Argentina by Niveiro and Popoff (2011). The phenotype of the Argentine collection is virtually indistinguishable from *P. cupulisnymphae* because both have similar habit, pileus color, contextual hypha diameter, and ecology.

As interpreted here, the known range of *P. cupulisnymphae* is extended considerably, assuming a wide Neotropical distribution, including, in addition to Brazil, putative records from Argentina, Bolivia, Guyana, Jamaica, Mexico, and Panama (Lowy 1959, 1971; Niveiro and Popoff 2011). Nevertheless, a broader taxon sampling of this species is obviously necessary in order to better understand the full range of its morphoanatomical variability and determine its ecological and biogeographic range more precisely.

Pseudohydnum viridimontanum C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 12, 13
MycoBank MB851359

Typification: BRAZIL. MINAS GERAIS: Camanducaia, Serra da Mantiqueira, Monte Verde, from decaying *Vernonanthura discolor* (Spreng.) H. Rob. wood, 29 Dec 2022, *C.*

Coelho-Nascimento & T. Comenale NCC266 (**holotype** FIFUNGI292), GenBank: ITS = OR625552, 28S = OR957373.

Diagnosis: Pseudohydnum viridimontanum is similar to *P. cupulisnymphae* but can be distinguished by its smaller and mostly short-stipitate basidiomata, smaller basidiospores (5.8–6.9 × 4.4–5.0 µm), presence of cylindrical to fusiform inflated elements in the spine trama, and absence of clamp connections. In addition, it was only found on *Vernonanthura discolor* wood.

Etymology: viridis (Latin) = green, *montanum* (Latin) = hill, mount, mountain; referring to the type locality, i.e., "Monte Verde" in Portuguese, which means Green Hill.

Morphology: Basidiomata small-sized, gelatinous when fresh, pileate, laterally stipitate or pseudostipitate, solitary or in imbricate clusters, at first as a globose to bell-shaped primordia, then expanding to orbicular, flabelliform or reniform. Pileus up to 35 mm diam, surface smooth to minutely rough, white (1A1) at first, then chalk white to grayish white (1B1 to somewhat paler) or grayish yellow (1B3, 3B2); margin sharp, even to crenate, sometimes undulating. Hymenophore white (1A1) to whitish, hydnoid. Hymenophore spines sharp-tipped, up to 0.8 mm long, 2.5–4 per mm, white (1A1) to translucent white. Stipe short to rudimentary. Context concolorous with the pileus, translucent appearance when wet, thick centrally and gradually thinner toward the margin, unchanging. Odor mild. Taste mild or pleasant.

Hyphal system monomitic, generative hyphae clampless, hyphae unchanged in KOH. Pileal surface subtrichodermial to trichodermial, composed of scattered fascicles of cylindrical hyphae (2.5–5.2 µm diam) that are colorless in mass and frequently septate, thin- to slightly thick-walled; terminal elements poorly differentiated. Contextual hyphae thin- to slightly thick-walled, hyaline, interwoven, frequently branched, commonly anastomosing, 1.5–5.0 diam. Spine tramal hyphae thin-walled toward the tips of the spines, slightly thick-walled toward the core, smooth, hyaline, branching, interwoven, 1.0–3.0 µm diam, sometimes forming intercalary and terminal inflated segments; intercalary inflated segments cylindrical to fusiform, 68–80 × 6.9–13.0 µm; terminal inflated segments long clavate, 40–85 × 9.0–14.0 µm. Hyphidia simple, thin-walled, hyaline, 1.0–2.7 µm diam at the apex, covering basidial cells. Basidia 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose, 9.2–12.0 × 8.1–10.6 µm; sterigmata up to 22 × 1.4–2.3 µm, mostly tubiform, usually slightly flaring at apex. Probasidia subglobose to short clavate, 8.8–9.0 × 6.5–7.5 µm, mostly multigutullate. Basidiospores [90/3/2] (5.0–)5.6–6.9(–7.3) × (3.9–)4.3–5.0(–5.5) µm [L_m = 6,2–6.5 µm; W_m = 4.6–4.7 µm; Q = (1.16–)1.23–1.53(–1.66); Q_m = 1.32–1.37], broadly ellipsoid to ellipsoid, rarely elongate, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, mostly germinating by repetition, sometimes by a germ tube conspicuously long, up to 28 µm long.

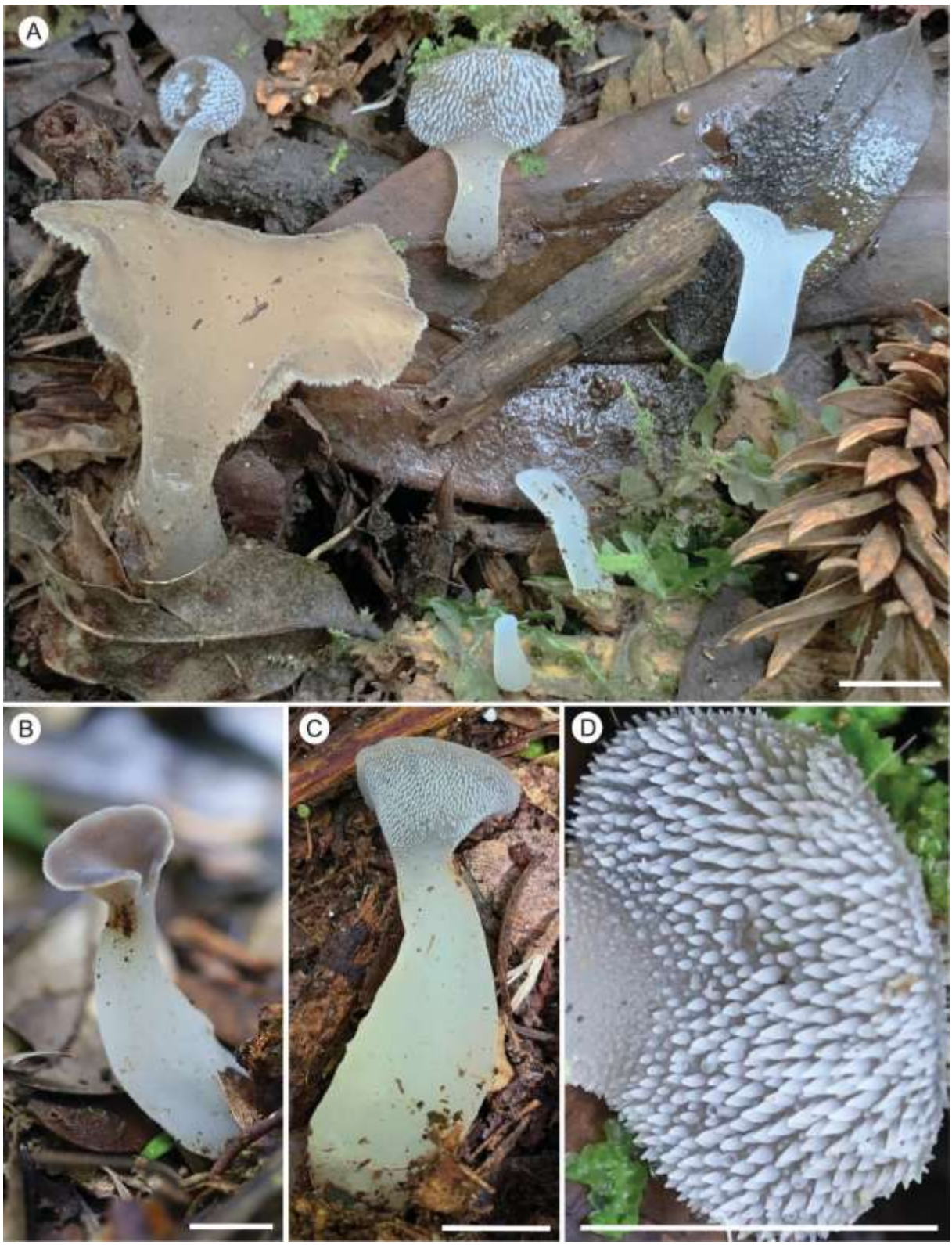

Figure 5. Basidiomata of *Pseudohydnum brasiliense*. A. MPD570 (holotype; FIFUNGI279). B–D. MPD572 (FIFUNGI280). Bars = 10 mm. Photographs: Mariana P. Drewinski.

Habitat and distribution: Growing on decayed wood of Vernonanthura discolor in upper montane mixed ombrophilous forest of Southeastern Brazil, at 1600 m a.s.l. Only known for the Brazilian state of Minas Gerais.

Other specimen examined: BRAZIL. MINAS GERAIS: Camanducaia, Serra da Mantiqueira, Monte Verde, from decaying *Vernonanthura discolor* wood, 5 Apr 2023, *C. Coelho-Nascimento & T. Comenale NCC273* (FIFUNGI293), GenBank: ITS = OR625553, 28S = OR957374.

Figure 6. Basidiomata of *Pseudohydnum brasiliense* with a fawn brown pileus surface. A–B. MIND.Funga0397 (FLOR 71400). C. MPD536 (FIFUNGI281). Bars = 10 mm. Photographs: A–B by MIND.Funga; C by Mariana P. Drewinski.

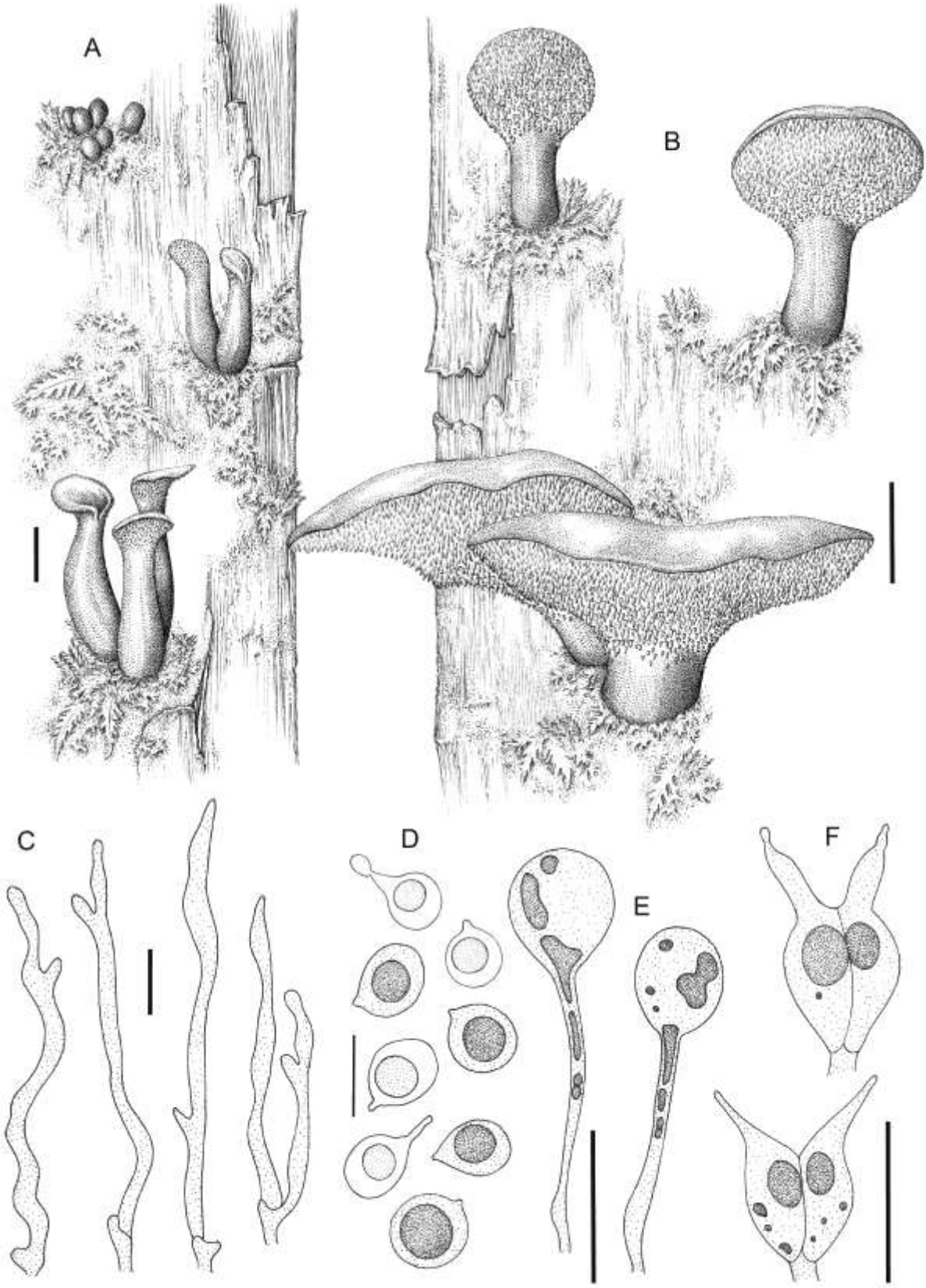

Figure 7. *Pseudohydnum brasiliense* (holotype; MPD570, FIFUNGI279). A. Young basidiomata. B. Mature basidiomata. C. Hyphidia. D. Basidiospores. E. Probasidia. F. Basidia. Bars: A–B = 10 mm; C–F = 10 μm.

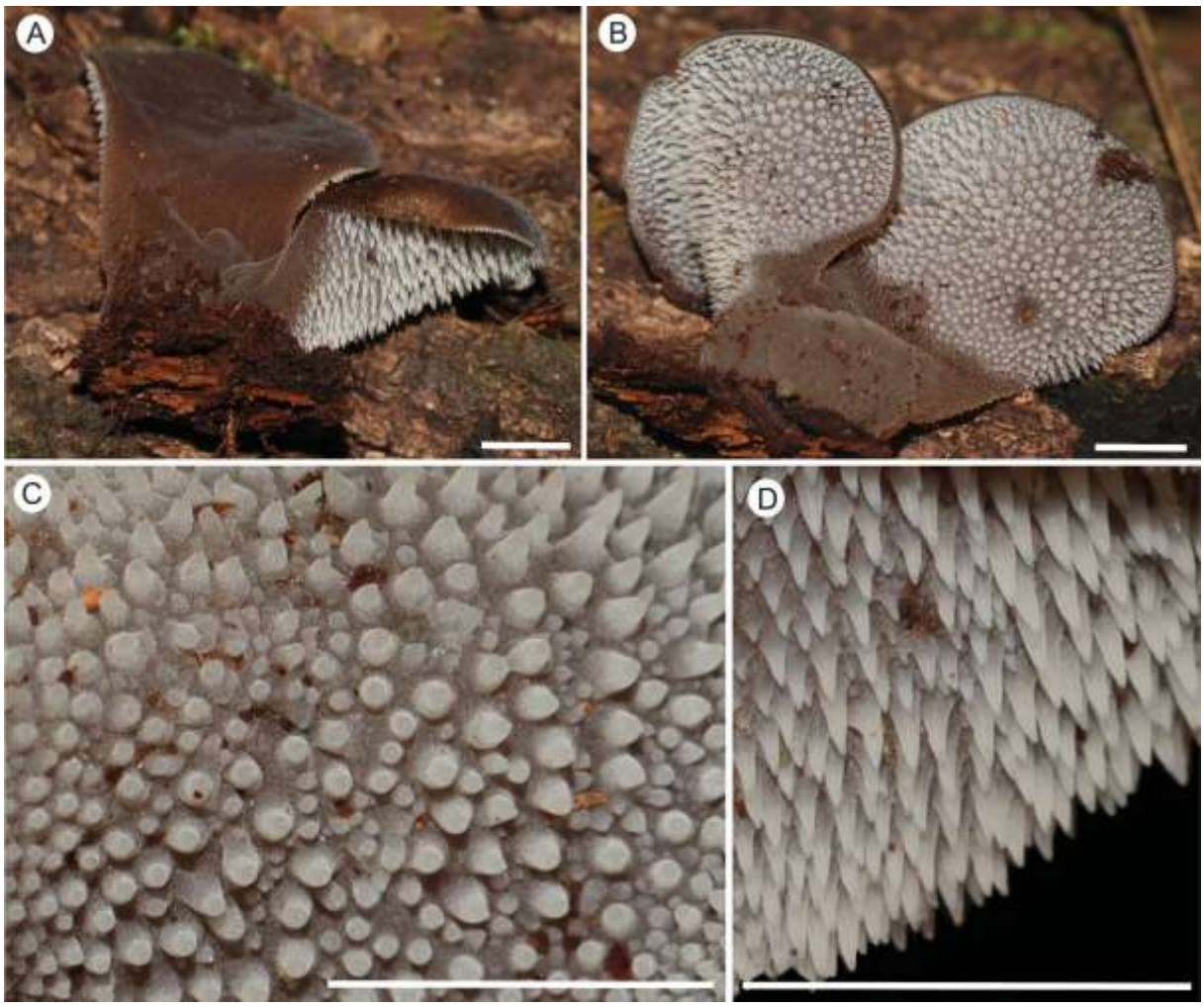

Figure 8. *Pseudohydnum brunneovelutinum* (holotype; AGS118/2022, FIFUNGI283). A–B. Basidiomata. C–D. Detail of the hymenophoral spines. Bars: 10 mm. Photographs: Alexandre G. dos Santos e Silva-Filho.

Notes: In the field, *P. viridimontanum* immediately evokes *P. cupulisnymphae* because of the white to grayish yellow pileus and occasional basidiomata with a rudimentary stipe. Both species also display similar hymenial to subhymenial anatomy. However, *P. cupulisnymphae* differs by its stouter and mostly sessile basidiomata, larger basidiospores (5.8–8 × 5.1– 7 µm) with a lower Q_m value (Q_m = 1.10–1.18), clamped generative hyphae, and the absence of cylindrical to fusiform inflated segments in spine trama. Furthermore, *P. viridimontanum* is also different by the ontogenetic configuration in young basidiomata because at first it forms a globose to bell-shaped basidiome that rapidly expands and assumes a mature flabelliform shape. In contrast, all young stages observed throughout *P.cupulisnymphae* collections involve a distinctly funnel-shaped or cupulate configuration.

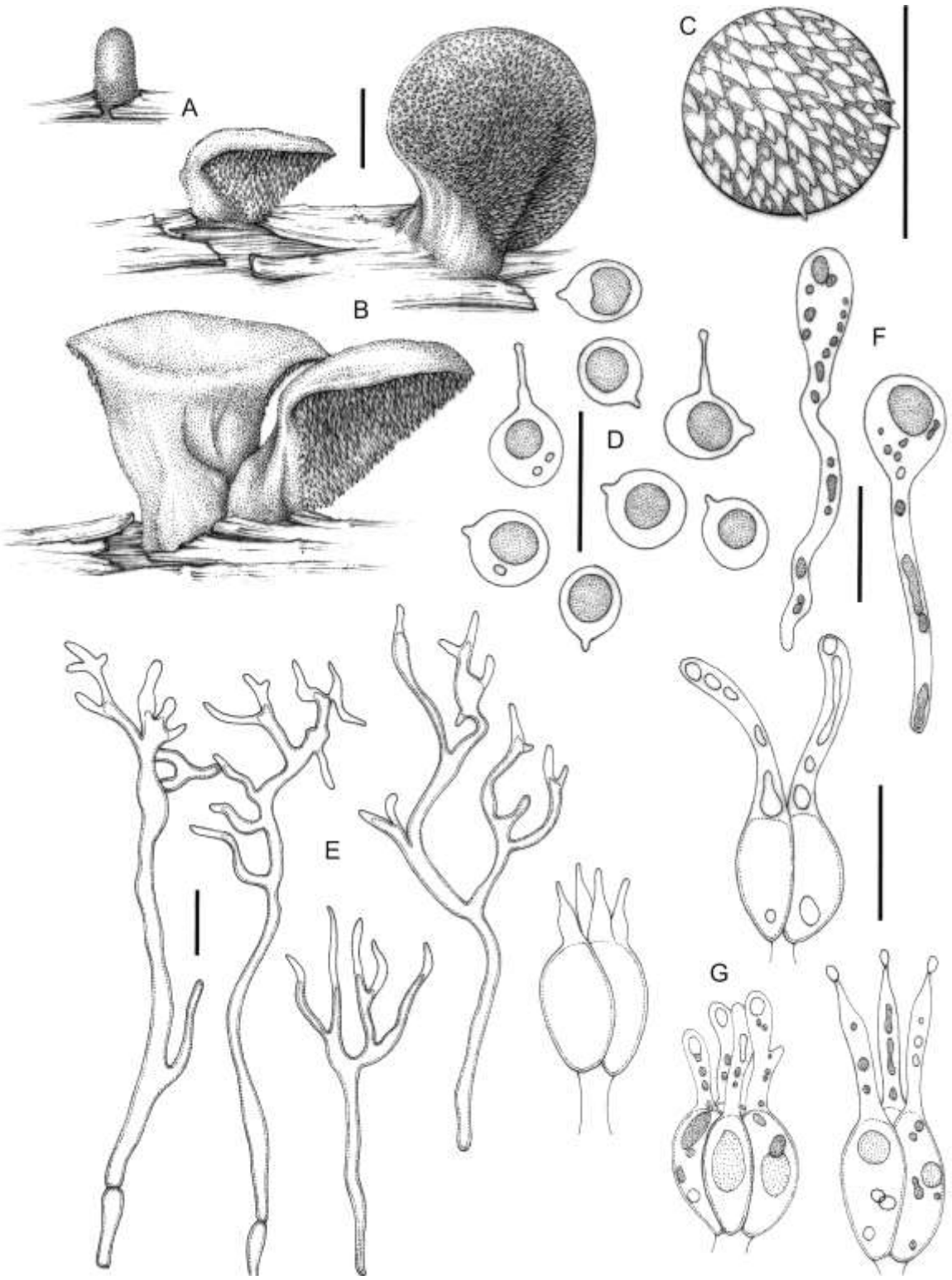

Figure 9. *Pseudohydnum brunneovelutinum* (holotype; AGS118/2022, FIFUNGI283). A. Young basidiomata. B. Mature basidiomata. C. Hymenophoral spines detail. D. Basidiospores. E. Hyphidia. F. Probasidia. G. Basidia. Bars: A–B = 10 μm; C = 5 mm; D–F = 10 μm.

Phylogenetically, based on the analyses restricted to *Pseudohydnum* species (FIG. 3), *P. viridimontanum* clusters with *P. umbrosum* in a well-supported clade in the BI analysis (lineage 1, BPP = 0.99) that is sister to the lineage around *P. translucens* (lineage 2). This phylogenetic relationship of *P. umbrosum* and *P. translucens* is consistent with the three-marker phylogeny

presented by Spirin et al. (2023) but is not consistent with the ITS+28S–based phylogeny presented by them, where the relationship between *P. umbrosum* and the clade around *P. translucens* is not resolved, with both clades placed in a polytomy. From a morphological perspective, *P. viridimontanum* barely resembles *P. umbrosum*. The latter is characterized by a dark-colored, fuscous-brown upper surface of pileus, denser hymenophoral spines (5–7 per mm), and larger basidiospores (7.6–9.8 × 6.0– 7.1 µm), as well as the lack of cylindrical to fusiform inflated segments in the spine trama (Spirin et al. 2023). *Pseudohydnum umbrosum* also has a very different ecology, growing on decayed conifer wood and so far known only from a boreal forest in the Russian Far East (Spirin et al. 2023).

Figure 10. Pseudohydnum cupulisnymphae. A–G. Basidiomata. H. Detail of the hymenophoral spines. A–C. MIND.Funga0040 (FLOR71289). D–F. NCC246 (FIFUNGI289). G–H. NCC247 (FIFUNGI 286). Bars = 10 mm. Photographs: A–C by MIND.Funga; D–H by Cristiano C. do Nascimento.

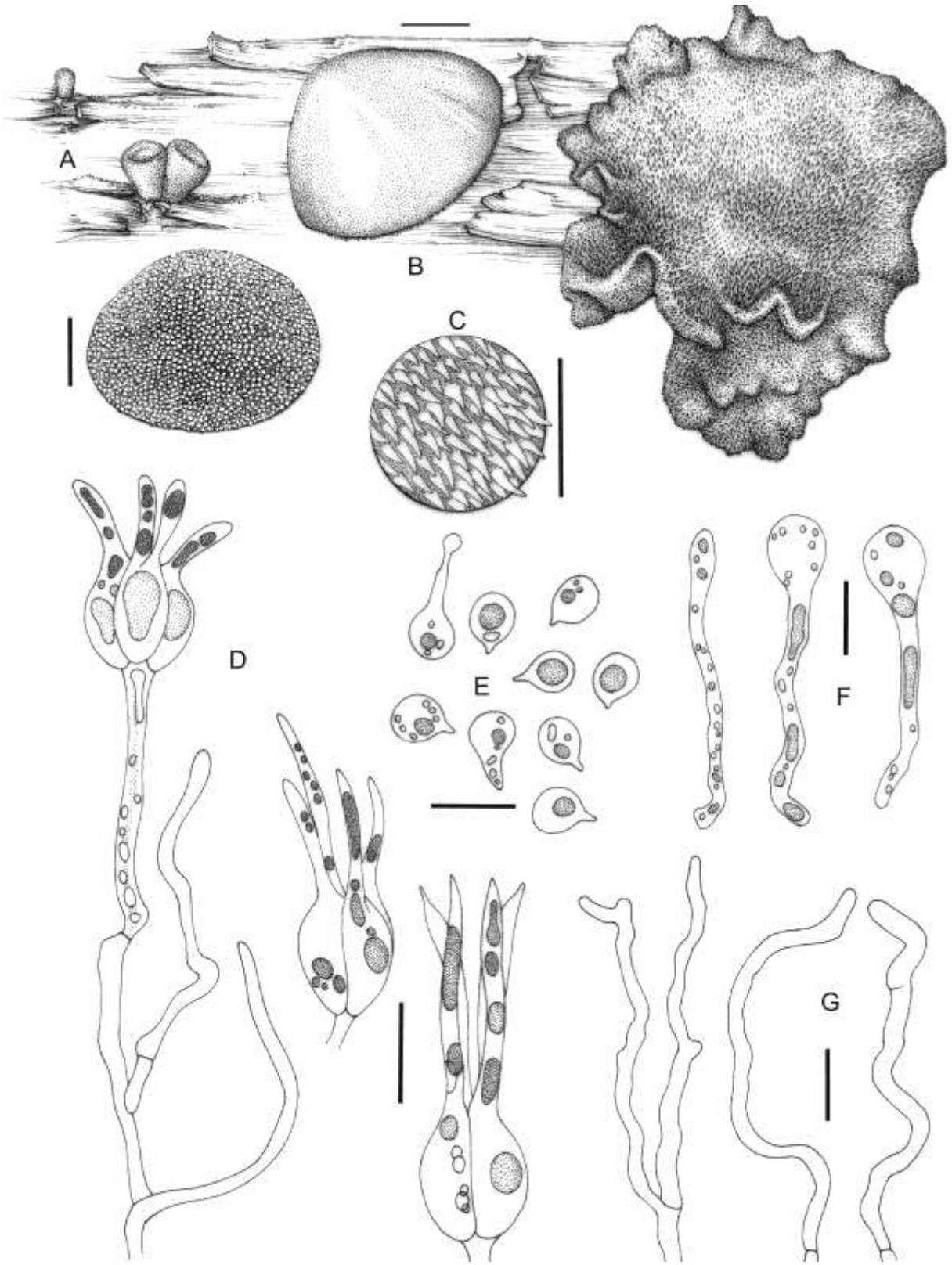

Figure 11. *Pseudohydnum cupulisnymphae* (holotype; NMJ420, FIFUNGI285). A. Young basidiomata. B. Mature basidiomata. C. Hymenophoral spines detail. D. Basidia. E. Basidiospores. F. Probasidia. G. Hyphidia. Bars: A–B = 10 mm; C = 5 mm; D–G = 10 μm.

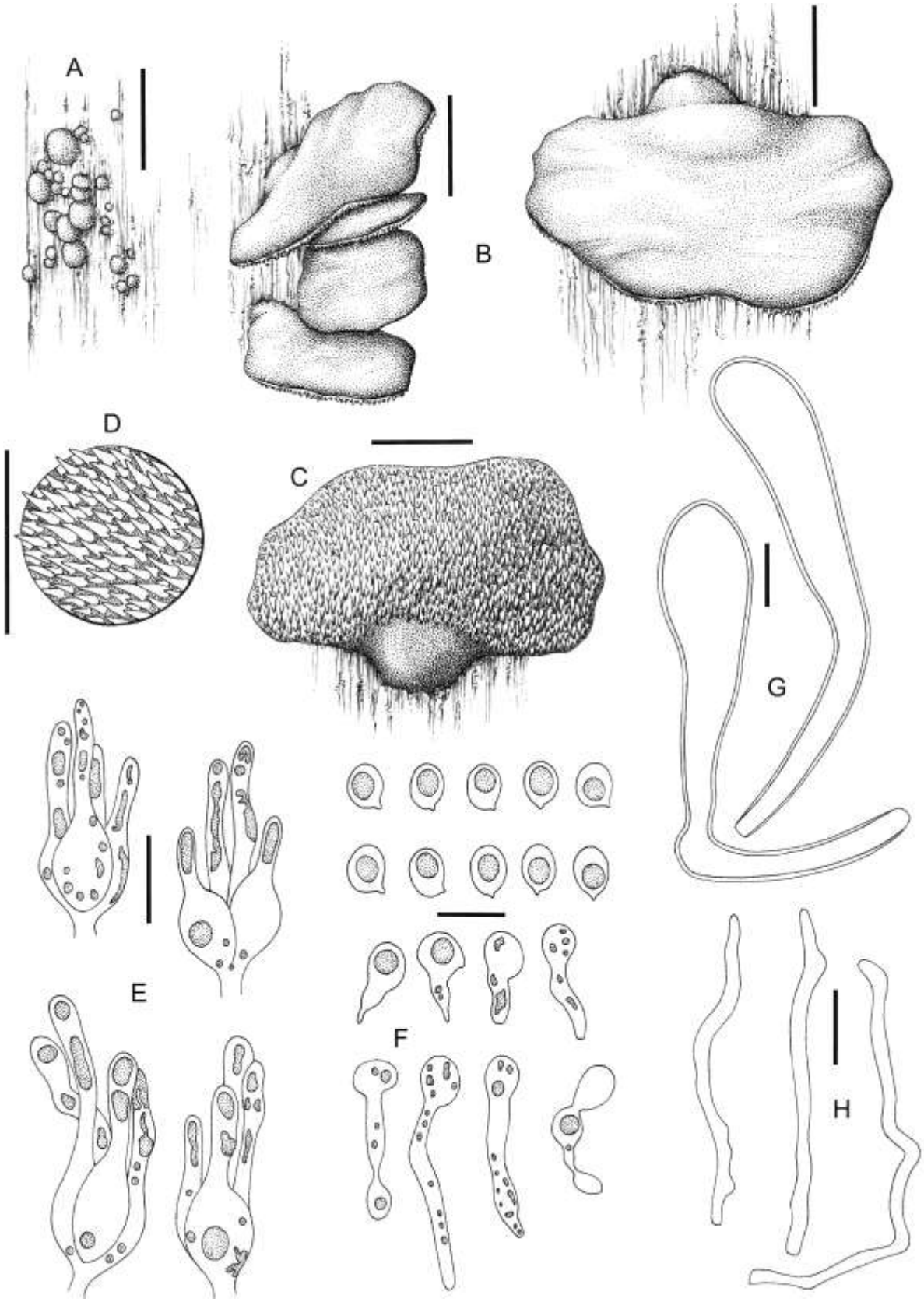

Figure 12. *Pseudohydnum viridimontanum* (holotype; NCC266, FIFUNGI292). A. Primordia and young basidiomata. B. Mature basidiomata, top view. C. Mature basidiomata, underside view. D. Hymenophoral spines detail. E. Basidia. F. Basidiospores. G. Terminal inflated elements from spine trama. H. Hyphidia. Bars: A–C = 10 mm; D = 5 μm; E–G = 10 μm.

Figure 13. Basidiomata of *Pseudohydnum viridimontanum*. A–C. NCC266 (holotype; FIFUNGI292). D–F. NCC273 (FIFUNGI293). Bars = 10 mm. Photographs: Thiago Comenale.

Pseudohydnum viridimontanum represents an edible wild mushroom that is harvested for consumption during the rainy season, in the type locality and surroundings; it is known as "cat's tongue auricularia" or "white auricularia." The local harvester (key informant) that collected *P. viridimontanum* type specimen (NCC266) reported the frequent consumption of *Pseudohydnum* basidiomata associated with *Vernonanthura discolor* decayed wood. Nevertheless, a systematic multiyear sampling of *Pseudohydnum* specimens in the referred area is necessary in order to confirm whether only *P. viridimontanum* occurs as a frequent species, or whether another similar species, such as *P. cupulisnymphae*, occurs and is also harvested wild. According to the aforementioned informant, *Pseudohydnum* basidiomata, which grow in large numbers, are collected during every rainy season by him and his family. The preparation for consumption is generally done by quickly sautéing the mushrooms, which are then used as a side dish, usually mixed with salad.

DICHOTOMOUS KEY FOR THE SEVEN CURRENTLY KNOWN TAXA OF PSEUDOHYDNUM FROM THE SOUTHERN HEMISPHERE

1. Basidiomata stipitate.....2

1′. Basidiomata sessile or rudimentarily stipitate.....3

2. Basidiomata confluent; basidiospores 5.5–6.5 × 4.8–5.7 µm.....*P. totarae*

2′. Basidiomata nonconfluent; basidiospores 6.4–9.7 ×–8.8 µm.....*P. brasiliense*

3. Pileus surface brown to grayish brown, dark brown, or reddish brown; spines sparse (0.5–1.5 per mm).....4

3′. Pileus surface whitish to grayish, grayish yellow, or pale pinkish orange; spines not sparse (≥2 per mm).....5

4. Basidiomata sessile; simple hyphidia present .....*P. orbiculare*

4′. Basidiomata short to rudimentary stipitate; dendrohyphidia present.....*P. brunneovelutinum*

5. Basidiospores ≥7.2 × 6.0 µm; on Eucalyptus and Nothofagus wood; restricted to Tasmania, Australia.....*P. tasmanicum*

5′. Basidiospores ≤7.0 × 6.0 µm; on angiosperm wood; Neotropica.....l6

6. Basidiospores subglobose to broadly ellipsoid (Qm = 1.18); inflated segments in spine trama absent; clamp connections present .....*P. cupulisnymphae*

6′. Basidiospores broadly ellipsoid to ellipsoid (Qm = 1.37); inflated segments cylindrical to fusiform present in spine trama; clamp connections absent.....*P. viridimontanum*

DISCUSSION

In the present study, four new species of *Pseudohydnum* were described from Brazil, in addition to 14 species recently introduced by Chen et al. (2020), Zhou et al. (2022, 2023), and Spirin et al. (2023). Even so, the species diversity in the genus seems to be much higher, especially in the Americas, as we could judge from the unique suite of macromorphological traits observed in a number of *Pseudohydnum* records retrieved from GBIF/iNaturalist platforms (FIG. 14). These records putatively represent undescribed species. Previous Neotropical records of collections identified as *P. gelatinosum* addressed here (viz., Courtecuisse and Lowy 1990; Lowy 1959, 1971; Möller 1895) morphologically fit with *P. cupulisnymphae* and/or *P. brasiliense* and allowed the distribution of both taxa to be expanded to other Neotropical areas. *Hydnum hirneoloides* Berk. & M.A. Curtis from Cuba, an old name associated with *P. gelatinosum*, is distinguished from all specific morphological concepts herein introduced. Further taxonomic studies should carefully reconsider this name when dealing with *Pseudohydnum* collections that display both short-stipitate/sessile basidiomata and a pale brownish pileus (Berkeley and Curtis 1868).

Figure 14. Macromorphological diversity of putatively undescribed *Pseudohydnum* species from the Americas. Data from GBIF/ iNaturalist. A–C, J–M, P. USA. D. Brazil. E. Canada. F, N, O. Ecuador. G–H. Mexico. I. Costa Rica. Photographs: A. ©Andrew Khitsun; B. ©Matt Pulk; C. © Elijah Disrude; D. © Felipe Bittencourt; E. © Igor Khomenko; F. © Damon Tighe; G. © Alan Rockefeller; H. © Hugo Basurto; I. © Andrey Loria; J. © Thom Worm; K. © Alan Rockefeller; L. © Colton Veltkamp; M. © Christian Schwarz; N, O. © Nolan Exe; P. © Jonathan Frank.

The systematic positioning of the four new described species is robustly supported by morphological and molecular results. The phylogenetic reconstruction based on three markers, ITS, 28S, and *RPB1*, allowed species recognition and also provided evidence for the recognition of eight subgroups corresponding to subclades/lineages (FIGS. 3, 1–8), as well as having been previously retrieved by Spirin et al. (2023). However, some intermediate and deep nodes had no significant statistical support, and there was a lack of resolution between the main recovered lineages. Other questions arising from the presented phylogenetic results will have to be tackled in a wider context with more and geographically wider samples and more loci. These include whether *P. gelatinosum* should be treated as a single transcontinental species or whether to attach any importance to the subtle morphological differences and independent phylogenetic position in order to consider the North American *P. gelatinosum* ssp. *pusillum* to be a distinct taxon. Both ITS+LSU and ITS+LSU+*RPB1* phylogenies clearly support the latter point; the *P. gelatinosum* ssp. *pusillum* sequences closely nest with other North American sequences labeled as "*P. gelatinosum*" and form a well-supported clade in the ML analysis (BS = 87%) (FIG. 3). Moreover, two collections also named "*P. gelatinosum*" were inferred as a well-supported clade (BPP = 1, BS = 100%) sister to *P. himalayanum* and which most likely represents an undescribed species.

Morphologically, the delimitation and recognition of the species described in detail here are unambiguous, although we assume that the limited availability of anatomical characters and the species' occasional high variability may represent an obstacle for morphological definition of *Pseudohydnum* species, mainly in terms of macromorphology. The only microscopic traits useful for species differentiation are those associated with the contextual and tramal hyphae, hyphidia, and basidiospores. The presence of clamp connections seems to be a common trait throughout all species of Pseudohydnum known so far. It was a difficult character to assess in some collections studied, especially in parts other than the hymenium. Remarkably, accurate analyses of all *P. viridimontanum* basidiomata failed to spot any clamp connections, showing that species are able to proliferate and reproduce without the necessity of clamp formation that putatively occurs in the *Pseudohydnum* lineage.

To provide a comprehensive framework for the *Pseudohydnum* species delimitation, we also propose to pay attention to some diagnostic macromorphological and ontogenetic traits. Macromorphologically, we regard the pileal surface, basidioma color(s), and, especially, the presence/absence of stipe of paramount taxonomic importance. Concerning the latter trait, basidiomata can range from distinctly stipitate to sessile. It is important to highlight that some species (e.g., *P. cupulisnymphae*) have mostly sessile basidiomata, producing occasional

basidiomata with a rudimentary stipe (pseudostipitate), whereas other species (e.g., *P. viridimontanum* and *P. gelatinosum*) are mostly short-stipitate but sometimes exhibit pseudostipitate or sessile basidiomata (Spirin et al. 2023). In contrast, some species seem to consistently produce stipitate basidiomata with either a short (e.g., *P. brunneovelutinum*) or a well-developed (e.g., *P. brasiliense*) stipe.

Ontogenetic characters are more difficult to assess because it is necessary to observe the basidiomata in their sequential developmental stages, from primordia to maturity. These traits were valuable to distinguish the species of *Pseudohydnum* described here. Specimens of *P. brasiliense*, for instance, always produce noncoalescent young basidiomata with a pseudoinfundibuliform pileus and a slightly inflated stipe (FIG. 5C), whereas the young stages of *P. cupulisnymphae* exhibit a cup-shaped pileus, with basidiomata often growing aggregated and forming a coalescent base (FIG. 9B). On the other hand, development in *P. brunneovelutinum* and *P. viridimontanum* is somewhat more direct, with intermediate stages that clearly resemble the basidiomata at maturity.

Species of *Pseudohydnum* occur all over the world, although the knowledge of species diversity and distribution remains rather fragmentary. Most of the known described species are from temperate to boreal Eurasia, suggesting that further studies are necessary in order to recognize *Pseudohydnum* diversity worldwide, especially in Neotropical habitats with high conservation value. Although the present study could be limited with regard to collecting sites and the number of collections studied, four new species from Brazil are described, and this provides an important step in the understanding of *Pseudohydnum* in the Neotropics and a basis for further development.

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Capítulo 4

A glimpse into the tropical underground: morphology and multigene phylogeny of true truffles (*Tuber*, Ascomycota) collections from Brazil reveal a new species and three new records

Artigo a ser submetido ao periódico **Mycologia**

A glimpse into the tropical underground: morphology and multigene phylogeny of true truffles (*Tuber*, Ascomycota) collections from Brazil reveal a new species and three new records

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ABSTRACT

Ectomycorrhizal fungi of the genus *Tuber* (Ascomycota) produce hypogeous ascomata commonly known as truffles, which play crucial ecological roles in forest ecosystems and are highly valued for their gastronomic and economic value. Despite extensive studies in temperate regions, the diversity and geographic distribution of *Tuber* species in tropical ecosystems, particularly in the Neotropics, remain largely understudied. In the present work, nine *Tuber* collections from Southern and Southeastern Brazil were analyzed using an integrative taxonomic framework. As a result, a novel species, *Tuber bianchettiformis* sp. nov., is formally described. Phylogenetic reconstructions based on the internal transcribed spacer (ITS) region and a concatenated multilocus dataset (ITS, LSU, *rpb2*, and *tef1-α*) consistently placed the new taxon within the Puberulum clade, where it forms a sister lineage to *T. cistophilum*. Morphologically, *T. bianchettiformis* is distinguished by its small-sized ascomata, which is whitish to brownish, with a somewhat thin pseudoparenchymatous peridium composed of subglobose, irregularly ellipsoid to polygonal cells (10–40 µm diam.), and sessile asci bearing 1–4 ellipsoid ascospores with a conspicuous reticulate alveolate ornamentation featuring broad meshes. In addition, three other phylogenetic species were identified: *T. brennemanii*, *T. lyonii*, and *T. maculatum*. The occurrence of *T. brennemanii* and *T. lyonii* represents the first confirmed records of these taxa outside North America, while *T. maculatum* is documented for the first time in the Brazilian funga.

INTRODUCTION

True truffles are fleshy ascomycetous fungi that primarily produce sequestrate ascomata. They belong to the genus *Tuber* P. Micheli ex F.H. Wigg, which is part of Tuberaceae (Pezizales), a family that also includes the lineages *Choiromyces* Vittad. and *Labyrinthomyces* Boedijn (Bonito et al. 2013, Graziosi et al. 2022, Mleczko et al. 2023). *Tuber* species typically thrive in well-drained soils rich in calcium and are often found in forested environments, particularly under coniferous trees (Mleczko et al. 2023). The ascomata of *Tuber* species are hypogeous, meaning they develop underground, which aids in their dispersal through animal consumption (Zambonelli et al. 2017, Mleczko et al. 2023, Rennick et al. 2023). Due to this belowground habitat of *Tuber* ascomata, its collection is challenging. Collectors, especially in Europe and North America, train dogs or pigs to assist in locating *Tuber*. The genus *Tuber* is characterized by its symbiotic relationships with a diverse range of host plants (e.g. Betulaceae, Cistaceae,

Fagaceae, Pinaceae, Salicaceae), forming ectomycorrhizal associations that are crucial for nutrient exchange, soil health, and overall ecosystem resilience (Bonito et al. 2010, Gryndler 2016, Leonardi et al. 2021a). While some *Tuber* species exhibit preferences for specific host plants, many are considered host generalists, capable of forming associations with multiple genera (Morris et al. 2008, Bonito et al. 2011, Leonardi et al. 2021a, Coleman et al. 2025). This adaptability allows *Tuber* to thrive in various habitats, from natural forests to cultivated truffle orchards (Bonito et al. 2010)

The genus *Tuber* is one of the most economically significant groups of truffles, with overall species diversity estimated to be between 150 and 230 species globally, making it one of the most diverse groups of ectomycorrhizal fungi (Bonito et al. 2013, Graziosi et al. 2022, Cseh et al. 2024, Hyde et al. 2024). Important species include *T. melanosporum* Vittad. (the black truffle) and *T. aestivum* Vittad. (the summer truffle), which are highly prized for their culinary value due to their unique blend of aromas and distinctive flavors (Graziosi et al. 2022, Coleman et al. 2025). *Tuber magnatum* Picco (the alba truffle), esteemed as "the food of the gods", has an intense aroma and luxurious culinary appeal, making it one of the most expensive foods globally and arguably also the most delicious truffle species (Allena & Bennett 2021, Čejka et al. 2023). *Tuber* aromatic qualities, which are largely attributed to volatile organic compounds (VOCs) released during the maturation of their ascomata. These volatiles not only attract animals that aid in spore dispersal but also mediate interactions with microorganisms and contribute to the truffles' distinctive flavors (Splivallo et al. 2011, 2015).

Tuber species recognition was traditionally reliant on a small number of morphological traits of the ascomata, such as peridium, asci and ascospores, leading to problems in accurate species delimitation and identification due to the common overlap of these traits (Mleczko et al. 2023). After the pivotal work of Bonito et al. (2010), we have witnessed a rapid increase in the number of newly described species, mainly due to the use of the ITS nrDNA barcode to aid species recognition. In addition, the application of multilocus phylogenetic analyses and next-generation sequencing has further facilitated a more comprehensive understanding of *Tuber* taxonomy and ecology (Vita et al. 2020). These methods allow for the identification of cryptic species and the exploration of genetic relationships that were previously difficult to resolve (Vita et al. 2020). In this context, at least 12 major clades have been identified within the genus, with most species diversity residing within the Rufum, Puberulum, and Maculatum clades (Bonito et al. 2013, Healy et al. 2016, Fan et al. 2016a, b).

Due to their strong dependence on ectomycorrhizal symbiosis, on edaphic conditions, and on zoochory, *Tuber* species exhibit a high continental or regional endemism (Bonito et al. 2010, Ori et al. 2021). The genus *Tuber* is primarily distributed across the Northern Hemisphere,

including Europe, Asia, North America, Central America, and Northern Africa (Mello et al. 2017, Leonardi et al. 2021a, Cseh et al. 2024). Several major *Tuber* clades such as Rufum, Melanosporum, Puberulum, Maculatum, and Macrosporum are widespread across these regions but maintain species-level endemism (Bonito et al. 2013). Other clades seem to cover rather limited areas: for example, representatives of the clades Gennadii and Multimaculatum are endemic to Europe, whilst the Japonicum and Gibbosum clades are endemic to Asia and North America, respectively (Bonito et al. 2013, Wang et al. 2024).

In South America, the diversity of *Tuber* taxa presents a unique biogeographical pattern characterized by limited endemic diversity and a predominance of exotic species (Bonito et al. 2013). For example, several *Tuber* species have been introduced to non-native habitats, such as *T. melanosporum*, *T. borchii* Vittad. (bianchetto truffle), and *T. aestivum*, which are successfully cultivated in Chile and Argentina (Zambonelli et al. 2015, Hall et al. 2017, Leonardi et al. 2021a). On the other hand, the Puberulum clade represents the only *Tuber* lineage naturally present in South America, where it has been identified in ectomycorrhizal associations with native tree species, such *Salix humboldtiana* Willd. and species of *Nothofagus* Blume (Bonito et al. 2013, Nouhra et al. 2013). This presence of representatives within this clade is attributed to either natural long-distance dispersal or anthropogenic introduction via the roots of imported trees brought by early settlers for forestry and landscaping purposes (Barroetaveña et al. 2006, 2010, Southworth et al. 2009, Guerin-Laguette et al. 2013, Pietras et al. 2013). Furthermore, white truffles belonging to the Maculatum clade, such as *T. maculatum* Vittad. and *T. floridanum* A.C. Grupe, Sulzbacher & M.E. Sm., have been found forming ectomycorrhizal associations with commercial pecan [*Carya illinoinensis* (Wangenh.) K. Koch] nut orchards in Uruguay and Brazil, most likely as the result of either the introduction of inoculated seedlings or the deliberate transfer of truffle-rich soil (Grupe II et al. 2018, Kuhar et al. 2024).

In an ongoing effort to understand biodiversity and ecology of wild edible mushrooms in Brazilian Atlantic domain (Zabin et al. 2024, Drewinski et al. 2024), and in collaboration with Pecan nut orchard and truffle dog owners, we have been surveying and identifying truffle collection in Southeastern and Southern Brazil since 2022. Here, we describe a new truffle species in the Puberulum clade based on morphological and multigene molecular phylogenetic evidence. In addition, the present study documents the first occurrence of *T. lyonii* Butters and *T. brennemanii* A.C. Grupe, Healy & M.E. Sm. outside of the North America, along with the first report of *T. maculatum* in Brazil.

MATERIALS AND METHODS

Collections.—Over the past three years, nine collections of *Tuber* were collected in Southern and Southeastern Brazil. In Southeastern Brazil, five collections were obtained from the municipal district of São Bento do Sapucaí, in the state of São Paulo (SP), a region encompassed by the Serra da Mantiqueira ecoregion. Additional two collections were sampled in this same ecoregion, specifically in the neighboring state of Minas Gerais (MG), in the municipality of Camanducaia. The remaining collections were found in association with *Carya illinoinensis* roots in commercial pecan orchards in the municipality of Cachoeira do Sul, state of Rio Grande do Sul (RS). More information about specific localities has been itemized under *Specimens examined*.

Fresh specimens from MG and RS were collected through a meticulous search of the soil surface or under leaf litter and soil using a hand rake, following the procedures outlined by Castellano et al. (1989, 2004). On the other hand, the SP specimens were found by trained truffle dogs. The dogs whose collections are included in this study are undergoing training to detect ripe truffles. Brazilian voucher specimens included in this study were dried using a hot air dehydrator (~45 °C), stored with allochroic silica gel in plastic bags, and accessioned into the fungarium IFungiLab (FIFUNGI) from the 'Instituto Federal de Educação, Ciência e Tecnologia de São Paulo' (IFSP), São Paulo, Brazil (Thiers 2024, continuously updated).

This study is according to the Brazilian legislation on access to biodiversity and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen # 0000000).

Morphological methods, notation and use of standards.—Details of the ascomata peridium and gleba were examined under a Zeiss Stemi 305 stereo microscope (Carl Zeiss AG, Oberkochen, Germany). Stereomicroscopy photographs were captured using a Zeiss Axiocam 208 color digital camera (Carl Zeiss AG).

For light microscopy, voucher specimens were hand-sectioned with a razor blade under a stereoscope or scratched with the point of a lancet, rehydrated for 15 min in 10% KOH, and then mounted in deionized water, 3% KOH, Melzer's reagent, or aqueous Congo red solution, each used separately. Slides were examined and photographed using a Zeiss Axioscope 5 (Carl Zeiss AG) compound microscope equipped with a Zeiss Axiocam 305 color digital camera. Measurements were performed on CZI (Carl Zeiss Image) images with the Zen 3.5 (Blue edition) software package (Carl Zeiss AG). Selected images were processed in Adobe Photoshop CC 2025 (Adobe Systems, São José, California) by adjusting brightness and contrast

and adding a greyish background. We characterized morphological traits previously reported in the literature as relevant for the group, including excipular tissues, ascospores, spore ornamentation, and asci (Korf 1973, Trappe et al. 2009, Healy et al. 2023). The average and standard deviation of at least 20–30 measurements from each feature were calculated. Given that the dimensions of ascospores in *Tuber* are known to vary between asci containing different numbers of ascospores, the length, width, and length/width ratio (Q) of the ascospores from each ascus type were measured and recorded separately (Sow et al. 2024). The mean spore ratio (Qm), calculated as the average of all individual Q values, was also provided.

Colors were generically named and then coded according to Kornerup & Wanscher (1978), with color plates noted in parentheses (e.g., 3A2).

Scanning electron microscopy (SEM).—For scanning electron microscopy, a piece of the apothecium was rehydrated in 3 % KOH, dehydrated using a series of ethanol concentrations (30, 50, 70, 90, 95, and 100%), and mounted on double-sided adhesive carbon tape on aluminium stubs, sputter-coated with 5 nm gold/palladium, and then viewed with a JEOL JSM – 6360LV Scanning Electron Microscope (JEOL Ltd., Tokyo, Japan) at 0.5–30 kV at the 'Centro de Microscopia da Universidade Federal de Minas Gerais' (CM-UFMG), Belo Horizonte, Brazil. To improve the sample conductivity silver colloidal paint was used. The images were digitally captured and then processed using Adobe Photoshop CC 2025 (Adobe Systems) and Topaz Photo IA 3 (Topaz Labs LLC., Dallas, Texas) for enhancements including upscaling, sharpening, and denoising.

DNA Extraction, PCR amplification, and sequencing.—For total genomic DNA isolation, the gleba tissue was excised from dried ascomata using a sterilized scalpel and tweezers. The procedure for the 'DNeasy Plant Mini Kit' (Qiagen, San Diego, California) DNA extraction kits were carried out according to the manufacturer's instructions with some modifications: following the addition of buffer AP1 and RNase A, the samples were subjected to an overnight incubation at 65 °C, and the applied volumes of elution buffer (AE) were 50 µL. DNA concentration of each sample was estimated using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Madison, Wisconsin).

Standard and touchdown polymerase chain reaction (PCR) protocols and fungal-specific and/or Tuberaceae-specific primers sets were used to amplify and sequence four loci: the nuclear rDNA internal transcribed spacer (ITS) region, the 28S rRNA gene (LSU), the second largest subunit of RNA polymerase II (*rpb2*), and the translation elongation factor 1-alpha (*tef1*-

α) region. The complete list of primers utilized for the amplification of these markers is provided in Table 1.

The PCRs were conducted on a Mastercycler Nexus GX2 thermal cycler (Eppendorf SE, Hamburg, Germany) according to the protocols delineated in Coelho-Nascimento et al. (2024). After visualization of positive PCR products on a 2% agarose gel with Safedye Nucleic Acid Stain (Cellco Biotech), amplicons were cleaned up prior to sequencing using QIAquick PCR Purification Kit (Qiagen) or PCR Purification Kit DPK-106 (Cellco Biotech) as per manufacturer's guidelines.

Table 1. Primer sequences used to amplify the four markers identified in the present study.

DNA region	Primer	Sequence (5' → 3')	Reference
ITS	ITS1F (Fw)	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns (1993)
	ITS1 (Fw)	TCCGTAGGTGAACCTGCGG	White et al. (1990)
	ITS4 (Rv)	TCCTCCGCTTATTGATATGC	White et al. (1990)
	ITS4-B (Rv)	CAGGAGACTTGTACACGGTCCAG	Gardes & Bruns (1993)
LSU	LR0R (Fw)	GTACCCGCTGAACTTAAGC	Vilgalys & Hester (1990)
	LR5 (Rv)	ATCCTGAGGGAAACTTC	Vilgalys & Hester (1990)
rpb2	fRPB2_5f (Fw)	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)
	RPB2 Tuber_f (Fw)	YAAYCTGACYTTRGCYGTYAA	Bonito et al. (2013)
	fRPB2-7cR (Rv)	CCCATRGCTTGYTTRCCCAT	Liu et al. (1999)
	RPB2 Tuber_r (Rv)	CRGTTTCCTGYTCAATCTCA	Bonito *et al*. 2013
tef1-α	EF1α Tuber_f (Fw)	AGCGTGAGCGTGGTATCA C	Bonito et al. (2013)
	EF1α Tuber_r (Rv)	GAGACGTTCTTGACGTTG AAG	Bonito et al. (2013)

Sanger sequencing was carried out at the 'Centro de Pesquisa sobre o Genoma Humano e Células-Tronco da Universidade de São Paulo' (CEGH-USP), São Paulo, Brazil or at Macrogen Korea (Seoul, South Korea) using the same primer set as those for PCR.

Taxon sampling and alignment for phylogenetic analyses.—DNA sequence chromatograms were manually checked for quality, edited where necessary, and low-quality ends pruned in Geneious Prime v2024.0.5 (Biomatters Ltd., Auckland, New Zealand) (https://www.geneious.com). Data for the studied specimens and the GenBank accession numbers of ITS, LSU, *rpb2*, and *tef1* sequences are presented in Table 3.

We compiled four datasets for phylogenetic analyses (Table 2). Datasets were aligned using MAFFT v.7490 as implemented in Geneious (Katoh et al. 2002, Katoh & Standley 2013), adjusting the direction of nucleotide sequences according to the first sequence and selecting the E-INS-i iterative refinement method. Subsequent manual curation was made using BioEdit v.

7.7.1 (Hall 1999) or AliView (Larsson 2014). Ambiguously aligned sites were eliminated, and the gaps were treated as missing data. The number of included taxa, length of aligned datasets, as well as the number of parsimony informative sites are given in Table 2.

Table 2. Datasets compiled for the present study.

	Dataset	Number of sequences	Alignment length	Number of parsimony informative sites	Figure number
I	**ITS** (Maculatum and Puberulum clades)	137	728	158	1
II	**ITS+LSU+*rpb2*+*tef1-α*** (Maculatum and Puberulum clades)	192	3122	722	2
III	**ITS** (Rufum clade)	62	840	162	3
IV	**ITS+LSU+*rpb2*** (Rufum clade)	122	2719	487	4

In the first dataset (ITS of Maculatum and Puberulum clades), we aimed to cover all major lineages within Maculatum and Puberulum clades. Additionally, two representatives of the closely related Latisporum clade were utilized, and three taxa of the Gibbosum clade were employed as outgroup, totaling 137 sequences. The second dataset (ITS+LSU+*rpb2*+*tef1-α* of Maculatum and Puberulum clades) primarily comprised combined four-loci sequences from Maculatum and Puberulum clades specimens that were employed in the first dataset but with a reduced number of specimens to maximize the number of markers, while maintaining the same number of terminals would result in a significant amount of missing data. The final dataset II represents 67 specimens (29 from the Maculatum clade, 32 from the Puberulum clade, three from the Latisporum clade, and three from the Gibbosum clade as outgroup).

The third dataset (ITS of Rufum clade) had the aim of showing the position of a Brazilian collection of *T.* cf. *lyonii* among the sequences from North America within the so-called *Tuber lyonii* complex in the Rufum clade (Leonardi et al. 2019, Sánchez-Ledesma et al. (2022), Sow et al. 2024). It contained a selection of ITS sequences representing the backbone of this clade including sequences from Asia, Europe, and North America. In addition, few representatives of the neighboring Melanosporum clade were included and sequences of *Choiromyces* species were used as outgroup. The final dataset III represents 62 specimens (56 from the Rufum clade, four from the neighboring Melanosporum clade, and two of *Choiromyces* species as outgroup). We compiled an additional dataset (dataset IV) that was subsamped from the dataset III to include mainly combined ITS+LSU+*rpb2* sequences. In addition, nine samples represented only by ITS+LSU were included in dataset IV to position the key taxa in the Rufum clade that

lacked the *rpb2* marker. The dataset IV was used to test additional markers to improve the phylogenetic accuracy for clades within the *T. lyonii* complex, although a complete and fully optimized matrix cannot be assembled due to the significant amount of missing data in markers other than ITS. The total number of specimens represented in the dataset IV was 35 (31 from the Rufum clade and four from the Melanosporum clade as outgroup).

To build the multilocus phylogenetic trees, individual gene alignments were concatenated using Mesquite v.3.70 (Maddison & Maddison 2023). The latter software was also used to convert the type and extension of the files between the different steps of the workflow.

Phylogenetic analyses.—The phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian inference (BI) via Cipres Scientific Gateway (https://www.phylo.org/portal2/home.action) (Miller et al. 2010). Maximum likelihood (ML) analyses and bootstrap support (BS) assessments were performed using RAxML-HPC v.8.2.12 on XSEDE (Stamatakis 2014) under the GTRCAT model with 25 per site rate categories, along with 1000 rapid bootstrap repetitions (Felsenstein 1985). Bayesian inference analyses (BI) for the reporting of Bayesian posterior probabilities (BPP) support for branches were performed using MrBayes on XSEDE (3.2.7a) (Huelsenbeck & Ronquist 2001, Ronquist et al. 2012). The best-fit nucleotide substitution model for each gene partition was determined with jModelTest2 v.2.1.6 on XSEDE (v. 3.2.6) (Posada & Crandall 1998) via the Cipres Science Gateway (Miller et al. 2010) based on the Bayesian Information Criterion (BIC). Four simultaneous Markov chains were run for 10 million generations and trees were sampled every 1,000th generation (resulting in 10,000 trees). The first 25% trees, which represent the burn-in phase of the analyses, were discarded, while the remaining ones were used for calculating the consensus tree and BPPs. To test the convergence and stability of the runs, the average standard deviation of split of frequencies (< 0.01) was evaluated in Tracer 1.6 (Rambaut et al. 2018).

Phylogenetic trees from ML analyses were visualized in FigTree v1.4.4 (Rambaut 2018)] and edited in CorelDRAW 2024 (Alludo, Ottawa, Canada) and Adobe PhotoShop CC 2024 (Adobe Systems, São José, California) for figure presentation and to complement with the BPP support values. Branches that received BS and BPP greater than or equal to 70% and 0.95, respectively, were considered to be significantly supported.

The final alignments for all datasets as well as the input data for the phylogenetic analyses (nexus blocks) and phylograms are stored in the '00000' web platform.

Table 3. List of species, sample numbers, collection localities, and GenBank accession numbers for ITS, nrLSU, *rpb2*, and *tef1-α* sequences included in the phylogenetic analyses. Sequences newly generated in this study are shown in bold. Sample numbers followed by (**H**) refer to holotype specimens.

Species/name	Sample no.	Locality	GenBank accessions			
			ITS	**nrLSU**	***rpb2***	***tef1-α***
			***Maculatum* phylogroup**			
T. arnoldianum	Sp46_RH1619 (**H**)	USA	KU186913	—	—	—
T. arnoldianum	167-98C	USA	KU186924	—	—	—
T. aztecorum	ITCV:993 (**H**)	Mexico	NR_159044	—	—	—
T. aztecorum	GG1109	Mexico	KY271790	—	—	—
T. castilloi	17f	Mexico	JF419240	—	—	—
T. beyerlei	JT32597 (**H**)	USA	HM485408	—	JQ954491	—
T. beyerlei	JT27167	USA	HM485409	—	—	—
T. bomiense	BJTC FAN467	China	OM265247	OM366206		OM649611
T. bomiense	SKM101 (**H**)	China	KC517480	—	—	—
T. brennemanii	MES-653 (**H**)	USA	MF611779	KY565252	MH084658	MH159203
T. brennemanii	RH 1279	USA	MF611789	KY565256	MH084660	MH159204
T. brennemanii	MES-2175	USA	MF611780	KY565254	MH084659	
T. brennemanii	BDIF1063	Brazil	**This study**	**This study**	**This study**	—
T. castilloi	ITCV 149 (**H**)	Mexico	NR_119865	NG_059939	—	—
T. dryophilum	RBG Kew K(M)133595	UK	EU784424	—	—	—
T. floridanum	MES-654 **(H)**	USA	MF611781	KY565259		MH159202
T. floridanum	MS475	USA	MF611782	—	MH032563	—
T. floridanum	TUBFLO_P14	Brazil	OP132946	—	—	—
T. foetidum	B-2489	Hungary	AJ557544	—	—	—
T. *guevarae* (Quercus EcM)	8D	Mexico	JF419252	—	—	—
T. hubeiense	HMAS 60233 (**H**)	China	KT067688	—	—	—
T. itzcuinzapotl	52-ZON (**H**)	Mexico	OR429351	—	—	—
T. lauryi	JT7701	USA	HM485366	—	—	—
T. linsdalei	L63 (**H**)	USA	HM485370	—	—	—
T. maculatum	BJTC FAN868	China	OM265274	OM366227	OM584283	OM649634
T. maculatum	BJTC FAN876	China	OM265278	OM366228	OM584284	OM649635
T. maculatum	BII-120	Hungary	AJ557519	—	—	—
T. maculatum	Vittad. TL5974	Denmark	AJ969627	—	—	—
T. maculatum	RBG Kew K(M)17936	UK	EU784428	—	—	—
T. maculatum	FLAS:MES-885	USA	MT156493	—	—	—
T. maculatum	NCC271	Brazil	**This study**	**This study**	**This study**	**This study**
T. maculatum	FLAB22	Uruguay	PP297667	—	—	—
T. maculatum	FLAB21	Uruguay	PP297668	—	—	—
T. mexiusanum	ITCV181	Mexico	HM485411	JF419293	—	JX022602
T. mexiusanum	ITCV3785	Mexico	HM485412	—	—	—
T. miquihuanense	ITCV885 (**H**)	Mexico	HM485414	JF419292	—	JX022603
T. murinum	48981	Germany	JF261371	—	—	—
T. pseudomaganatum	BJTC FAN163 (**H**)	China	JQ771192	KP276192	OM584219	KP276208
T. pseudomaganatum	BJTC FAN391	China	OM265244	OM366195	OM584258	OM649600
T. scruposum	CMI-UNIBO 2201	Armenia	DQ011845	—	—	—
T. scruposum	CMI-UNIBO 2192	Armenia	DQ011846	—	—	—
T. shearii	OSC51052	USA	HM485389	JF419280	JQ954521	—

T. shearii	JT12498	USA	GQ221450	—	—	—
T. rapaeodorum	ZB85/11	Romania	JF261404	JF261370	—	—
T. rapaeodorum	B-1360	Hungary	AJ557522	—	—	—
T. rapaeodorum	JT13999	USA	HM485384	—	—	—
T. rapaeodorum	RBG Kew K(M)7705	UK	EU784430	—	—	—
T. microverrucosum	BJTC FAN142 (**H**)	China	JN870099	—	—	—
T. walkeri	RH754 (**H**)	USA	JF419265	—	—	—
T. walkeri	RH794	USA	JF419258	JF419308	—	—
T. whetstonense	JT5875	USA	JF419243	—	—	—
T. wumengense	BJTC FAN218A (**H**)	China	KT067682	KT067707	OM584230	KT067714
T. wumengense	BJTC FAN292	China	KT067683	KT067709	OM584239	KT067716
Tuber sp.	ITCV180	Mexico	HM485405	—	—	—
Tuber sp.	RH965	USA	JF419257	—	—	—
Tuber sp.	HZ1_RH1645	USA	KU186916	—	—	—
Tuber sp.	OTA:70451	New Zealand	OQ064897	—	—	—
Tuber sp. (*Pinus tabuliformis* EcM)	DGGE gel band 10	China	KF032601	—	—	—
Tuber sp. (*Pinus densiflora* EcM)	ZE14	China	GU134516	—	—	—
Tuber sp. (*Carya illinoinensis* EcM)	14d	USA	GQ379736	—	—	—
Tuber sp. (*Notholithocarpus densiflorus* EcM)	D68	USA	DQ273348	—	—	—
Tuber sp. (*Quercus robur* EcM)	BP98695	Hungary	DQ355256	—	—	—
Tuber sp. (*Quercus* EcM)	51E	Mexico	JF419268	—	—	—
Tuber sp. (*Quercus* EcM)	bg15c	USA	DQ974800	—	—	—
Tuber sp. (*Quercus* EcM)	48D	Mexico	JF419253	—	—	—
Tuber sp. (*Tilia cordata* EcM)	J56	Estonia	AJ534706	AJ534706	—	—
			***Puberulum* phylogroup**			
T. anniae	BJTC FAN640	China	OM286868	OM366215	OM584274	OM649620
T. anniae	JT13209 (**H**)	USA	HM485338	JQ925680	—	JX022567
T. anniae	JT22695	USA	HM485339	JQ925681	—	JX022568
T. asa	M1828	Italy	HM485341	—	—	—
T. bianchettiformis	NCC276	Brazil	**This study**	**This study**	**This study**	**This study**
T. bianchettiformis	NCC277	Brazil	**This study**	**This study**	**This study**	**This study**
T. bianchettiformis	NCC272 (H)	Brazil	**This study**	**This study**	**This study**	**This study**
T. bianchettiformis	CT01	Brazil	**This study**	**This study**	**This study**	—
T. bianchettiformis	CT02	Brazil	**This study**	**This study**	**This study**	—
T. bianchettiformis	483-Sulzbacher	Brazil	**This study**	**This study**	**This study**	**This study**
T. bonitoi	JT32421 (**H**)	Mexico	KT897472	KT897472	—	—
T. bonitoi	GO-2009-221	Mexico	KC152256	—	—	—
T. borchii	GB1/GB32	Italy	FJ809852	FJ809799	JQ954492	JX022571
T. borchii	BJTC FAN217	New Zealand	KT067681	KT067706	OM584229	KT067717
T. borchii	GB62	Italy	HM485342	—	—	—
T. borchii	CBS 272 72	Italy	KT215193	KT215193	—	—
T. brunneum	JT33835 (**H**)	Mexico	KT897474	KT897474	—	—
T. brunneum	JT33836	Mexico	KT897475	KT897475	—	—
T. californicum	JT28058	USA	HM485346	JQ925685	JQ954496	JX022574
T. californicum	JT22590	USA	HM485351	—	—	—
T. cistophilum	AH39275 (**H**)	Spain	JN392231	JN392293	—	—
T. dryophilum	GB37	Italy	HM485354	JQ925688	JQ954501	JX022578

T. dryophilum	GB35	Italy	JQ925644	—	—	—
T. huizeanum	BJTC FAN186 (**H**)	China	JQ910651	OM366170	—	OM649575
T. huizeanum	BJTC FAN313	China	KT067684	KT067691	OM584245	KT067715
T. incognitum	JT19301 (**H**)	Mexico	GQ221447	—	—	—
T. incognitum	ECMPCui_17C5	Mexico	MH174661	—	—	—
T. lijiangense	HKAS 52005 (**H**)	China	KF805727	—	—	—
T. lijiangense	GB312	China	HM485402	—	—	JX022573
T. maculatum	GO-2008-144	Mexico	KJ595014	—	—	—
T. microsphaerosporum	BJTC FAN152 (**H**)	China	KF805726	—	—	—
T. oligospermum	AH38984	Spain	JN392261	JN392320	—	—
T. oligospermum	AH37783	Spain	JN392260	JN392324	—	—
T. pacificum	OSC 62156	USA	EU834223	—	—	—
T. pacificum	OSC 62159	USA	EU837241	KT968651	—	—
T. pseudoseparans	JT33778 (**H**)	Mexico	KT897480	KT897480	—	—
T. pseudoseparans	JT33774	Mexico	KT897481	KT897481	—	—
T. puberulum	TL11885	Denmark	AJ969626	AJ969626	—	—
T. puberulum	TL3857	Denmark	AJ969625	AJ969625	—	—
T. separans	JT32463	Mexico	GQ221448	—	—	—
T. separans	JT6499	USA	HM485388	—	—	—
T. sinoborchii	BJTC FAN169	China	OM286800	OM366166	OM584222	OM649572
T. sinoborchii	BJTC FAN171	China	OM286802	OM366167	OM584223	OM649573
T. sinopuberulum	BJTC FAN157 (**H**)	China	JQ690073	—	—	—
T. sinosphaerosporum	BJTC FAN135 (**H**)	China	JX092086	KP276195	OM584217	OM649569
T. sinosphaerosporum	BJTC FAN136	China	JX092087	KP276196	—	KP276211
T. sphaerospermum	AH39190	Spain	JN392246	JN392305	—	—
T. sphaerospermum	AH39184	Spain	JN392247	JN392306	—	—
T. sphaerosporum	JT12487	USA	FJ809853	—	—	—
T. sphaerosporum	JT19772	USA	FJ809854	—	—	—
T. sphaerosporum	OSC75864	USA	HM485390	—	—	—
T. tequilanum	JT33796 (**H**)	Mexico	KT897482	KT897482	—	—
T tequilanum	JT33803	Mexico	KT897484	KT897484	—	—
T. vesicoperidium	BJTC FAN155 (**H**)	China	JQ690071	—	—	—
T. vesicoperidium	BJTC FAN156	China	JQ690072	—	—	—
T. zhongdianense	wang0299 (**H**)	China	DQ898187	—	—	—
Tuber sp.	GO-2010-013	Mexico	KC152267	—	—	—
Tuber sp.	KA-2010	Japan	AB553464	—	—	—
Tuber sp.	K06FC8T13a	New Zealand	GQ267493	—	—	—
Tuber sp. (EcM)	PS8	Latvia	KT182910	—	—	—
Tuber sp. (*Pinus nigra* EcM)	5717 h5	USA	KU186939	—	—	—
Tuber sp. (*Corylus avellana* EcM)	P-11-2-19	Canada	KF742744	—	—	—
Tuber sp. (*Pinus densiflora* EcM)	ZE26	China	GU134510	—	—	—
Tuber sp. (*Tilia amurensis* EcM)	R3	China	KJ173913	—	—	—
Tuber sp. (*Corylus avellana* EcM)	M-11-43-36	Canada	KF742730	—	—	—
		***Rufum* phylogroup**				
T. caryophilum	st3 (**H**)	Mexico	MZ092919	OK642397	—	—
T. candidum	SOC 727	USA	AY830856	—	—	—
T. candidum	FLAS:F-60824	USA	MT156526	—	—	—

T. crassitunicatum	BJTC FAN465	China	MH115295	OM366205	OM584268	—
T. cumberlandense	FLAS-F-68201 (**H**)	USA	PP337301	PQ642898	—	—
T. cumberlandense	FLAS:F-71137	USA	PP337339	PQ771171	PP872520	—
T. cumberlandense	FLAS:F-71135	USA	PP337338	PQ771170	PP872519	—
T. ferrugineum	MUB:Fung-0972	Spain	MN962719	—	—	—
T. furfuraceum	HU061120C	Taiwan	FJ176920	—	—	—
T. huidongense	BJTC FAN101	China	OM311172	OM366156	OM584208	—
T. lannaense	SDBR-CMU-MTUF006 (**H**)	Thailand	KT758730	KU207732	—	—
T. liaotongense	BJTC FAN550	China	MH115302	OM366213	OM584272	—
T. liaotongense	BJTC FAN718 (**H**)	China	MH115303	MH115304	OM584276	—
T. lishanense	BJTCFAN683	China	MH115305	MH115306	OM584275	—
T. luomae	OSC:151373	USA	MH142475	—	—	—
T. luomae	OSC:148706	USA	MH142474	FJ809812	—	—
T. lyonii	BDB869	Brazil	**This study**	**This study**	**This study**	—
T. lyonii	JV544	USA	PP947705	—	—	—
T. lyonii	JV544_Tuber_cf_lyonii	USA	PP856165	PP856165	—	—
T. lyonii	GBTlyon2H12	—	PP947709	—	—	—
T. lyonii	GACalhounCountyfb	USA	EU268567	—	—	—
T. lyonii	GB108	USA	FJ748910	—	—	—
T. lyonii	JT5565	USA	FJ809883	—	—	—
T .lyonii	GA21	USA	—	JQ925698	JQ954510	—
T. lyonii	OSC58244	USA	—	JQ925699	JQ954511	—
T. lyonii	FLAS:MES-2192	USA	MT156509	—	—	—
T. lyonii	GB 112	USA	EU394704	EU394704	—	—
T. malacodermum	JT32319	Spain	FJ809889	JQ925702	JQ954514	—
T. melosporum	AH31737	Spain	JX402095	JN392202	—	—
T. nitidum	BM105	Spain	FJ809885	FJ809807	JQ954517	—
T. piceatum	HMAS:97125 (**H**)	China	MH115318	—	—	—
T. pustulatum	LUGO:ECC17072701	Spain	MW376716	—	—	—
T. rufum	—	Italy	AY940646	—	—	—
T. rufum	1480	Italy	EF362476	—	—	—
T. rugosum	BR64 (**H**)	USA	MW579343	MW579328	MW584657	—
T. rugosum	BR159	USA	MW579345	MW579330	MW584659	—
T. rugosum	T15106	Canada	HM485428	—	—	—
T. spinoreticulatum	U188 (**H**)	USA	FJ809884	FJ809815	JQ954527	—
T. subglobosum	BJTC FAN222	China	KF002728	OM366175	OM584232	—
T. subglobosum	BJTC FAN153 (**H**)	China	JX267043	—	—	—
T. quercicola	SOC 733	USA	AY918957	—	—	—
T. taiyuanense	BJTC FAN225	China	MH115325	OM366176	OM584233	—
T. taiyuanense	BJTC FAN164	China	OM311182	OM366164	OM584220	—
T. texense	JT15162	USA	HM485391	—	—	—
T. theleascum	AQUI 9729 (**H**)	Mexico	NR_164592	MK211312	—	—
T. theleascum	AQUI 9730	Mexico	MK211284	MK211313	—	—
T. umbilicatum	BJTC FAN317	China	OM311216	OM366185	OM584248	—
T. umbilicatum	BJTC FAN344	China	OM311220	OM366188	OM584251	—
T. wenchuanense	BJTC FAN833	China	OM311256	OM366222	OM584280	—
Tuber sp. 79	ITCV910	Mexico	JQ925649	JQ925710	JQ954522	—
Tuber sp. 64	FLAS-F-61989	USA	PP947707	—	—	—
Tuber sp. 64	FLAS-F-65653	USA	PP947708	—	—	—

Tuber sp. 64	RH8310775	USA	HM485425	—	—	—
Tuber sp. 65	ITCV884	Mexico	HM485426	—	—	—
Tuber sp. 65	ITCV908	Mexico	JQ925648	JQ925709	—	—
Tuber sp. 119	FLAS:F-68195	USA	PP337295	—	—	—
Tuber sp.	FLAS-F-71066	USA	PP947706	—	—	—
Tuber sp.	JT6003	USA	FJ809887	—	—	
***Latisporum* phylogroup**						
T. baoshanense	BJTC FAN400	China	OM256791	OM366197	OM584260	OM649602
T. latisporum	BJTC FAN125	China	KT067676	KT067695	OM584214	KT067725
Tuber sp.	KA-2010 K184	Japan	AB553458	AB553523	AB553563	AB553543
***Melanosporum* phylogroup**						
T. melanosporum	GB200	Italy	FJ748904	JQ925703	JQ954515	—
T. longispinosum	K225	Japan	AB553414	AB553518	AB553558	—
T. yigongense	BJTC FAN731 (**H**)	China	MF663714	OM366216	—	—
T. variabilisporum	BJTC FAN 362 (**H**)	China	OM287845	OM366190	OM584253	—
***Gibbosum* phylogroup (Outgroup I)**						
T. gibbosum	JT30580	USA	FJ809868	FJ809868	JQ954506	JX022585
T. oregonense	GB284 (**H**)	USA	FJ809874	FJ809874	JQ954518	—
T. bellisporum	JT11679 (**H**)	USA	FJ809855	FJ809855	JQ954490	—
***Choiromyces* (Outgroup II)**						
Choiromyces alveolatus	MES97	USA	HM485332	JQ925660	JQ954470	—
Choiromyces meandriformis	RH691	USA	HM485330	—	JQ954471	—

RESULTS

The ITS, LSU, *tef1-α*, and *rpb2 markers* are commonly used in *Tuber* phylogenetics, as they provide reliable information across different taxonomic levels. In this study, a total of 32 new sequences were generated (9 ITS, 9 LSU, 9 *rpb2*, and 5 *tef1-α*) (Table 3) from nine samples of four species.

The clustering of the species in both ML and BI phylogenetic trees was supported with congruent topologies. Some topological differences in the deeper branches were, however, noticed. The resulting ML phylogenetic tree based on our analyses is shown in Figures 1–4. Support values were shown on the branch when the ML bootstrap support (BS) ≥ 70 % and Bayesian posterior probabilities (BPP) ≥ 0.95.

Phylogenetic reconstructions of the Rufum clade based on ITS (dataset III) and multilocus (dataset IV) datasets (Figures 3, 4) placed the Brazilian specimen BDB869 in a strongly supported clade (BS = 95–100%; BPP = 0.99–1.00) together with reference sequences of *T. lyonii* from the USA. This lineage is here interpreted as *T. lyonii* s. str., nested within the previously circumscribed *T. lyonii* species complex (Figures 3, 4; Leonardi et al. 2019, Sánchez-Ledesma et al. 2022, Rennick et al. 2023, Sow et al. 2024). This complex also

comprises *T. caryophilum* Sánchez-Ledesma, Guevara-Guerrero & Gar.-Orijel from Mexico, *T. texense* Heimsch from the USA, *T. theleascum* M. Leonardi, A. Paz, G. Guevara & Pacioni from Mexico, and *Tuber* sp. 64 from the USA, which formed a sister lineage to *T. lyonii* s. str. in the ITS analyses (Figure 3), although this relationship was strongly supported (BS = 100%; BPP = 1.00) only in the multilocus analyses, which support the sister relationship of *T. lyonii* with *T. caryophilum* and *T. theleascum* (Figure 4).

Phylogenetic inference of the Maculatum clade (datasets I and II) revealed the occurrence of two *Tuber* species in Brazil, *T. maculatum* and *T. brennemanii* (Figures 1, 2). The Brazilian specimen of *T. maculatum* (NCC271) was nested within a fully supported clade (BS = 100%; BPP = 1.00) in both ITS and combined analyses (Figures 1, 2), comprising conspecific sequences from Eurasia, New Zealand, Uruguay, and the USA, and thus confirming its assignment to *T. maculatum*. In the multilocus phylogeny (Figure 2), *T. maculatum* was recovered as part of a strongly supported lineage (BS = 93%; BPP = 0.99) comprising *T. arnoldianum* Healy, Zurier & Bonito, *T. floridanum*, *T. shearii* Harkn, and *T. walkeri* Healy, Bonito & G. Guevara, all from the USA, together with *T. pseudomagnatum* L. Fan from China and an unidentified sample (J56) from Estonia. This lineage was resolved in the combined analyses (Figure 2) as sister to a well-supported clade (BS = 100%; BPP = 1.00) in which the Brazilian specimen (BDIF1063) of *T. brennemanii* was positioned. This specimen clustered with reference sequences of *T. brennemanii* from Mexico (Figure 1) and the USA (Figure 1, 2) with strong support (BS = 97–100%; BPP = 1.00). In the ITS-based phylogeny (Figure 1), *T. brennemanii* was instead recovered as sister (BS = 73; BPP = 0.95) to a monophyletic assemblage composed exclusively of taxa from Mexico, including *T. aztecorum* G. Guevara, Bonito & M.E. Sm., *T. castilloi* G. Guevara, Bonito & Trappe, *T. guevarae* Bonito & Trappe, and an unidentified specimen (ITCV180).

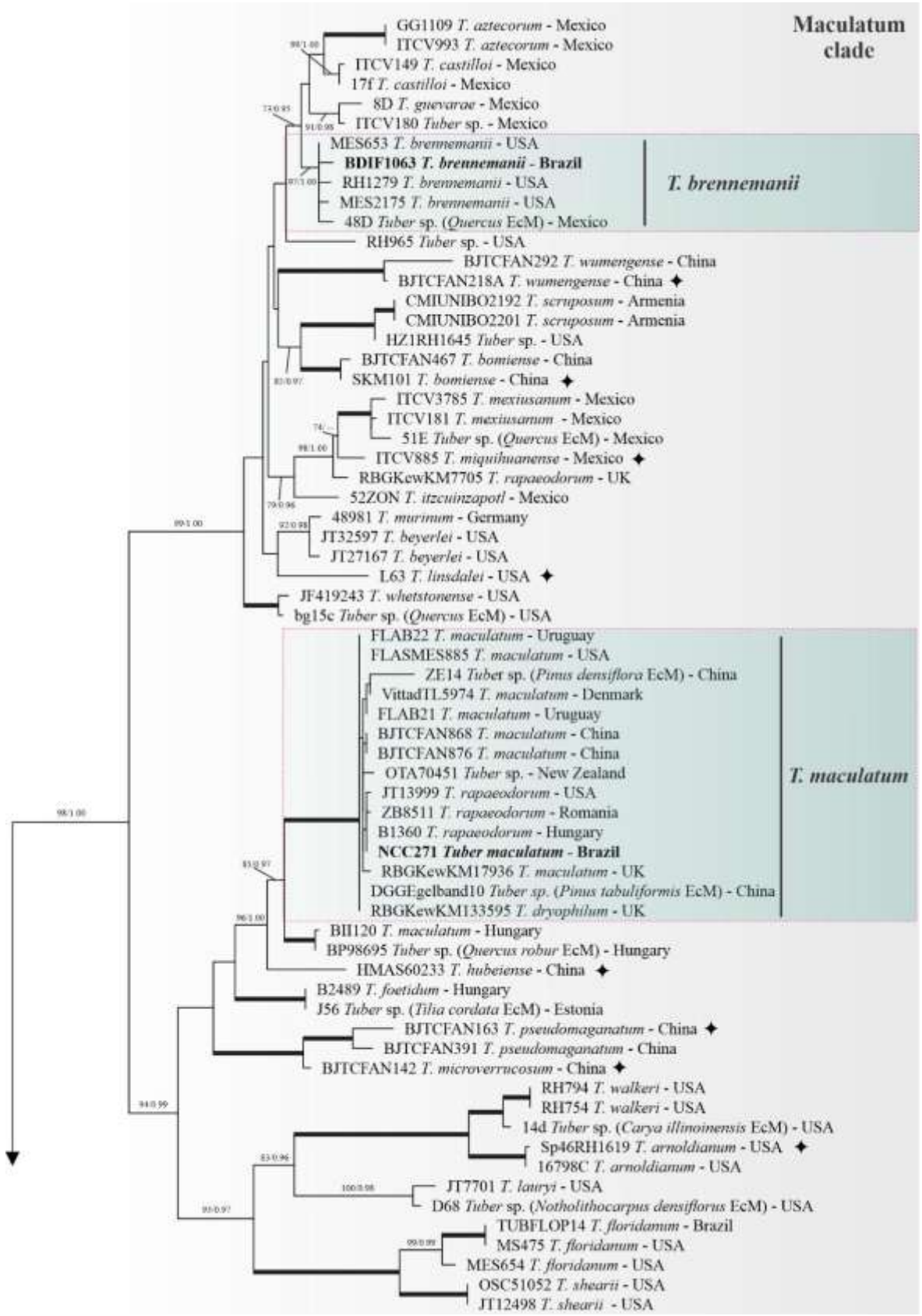

Figure 1. *Cont.*

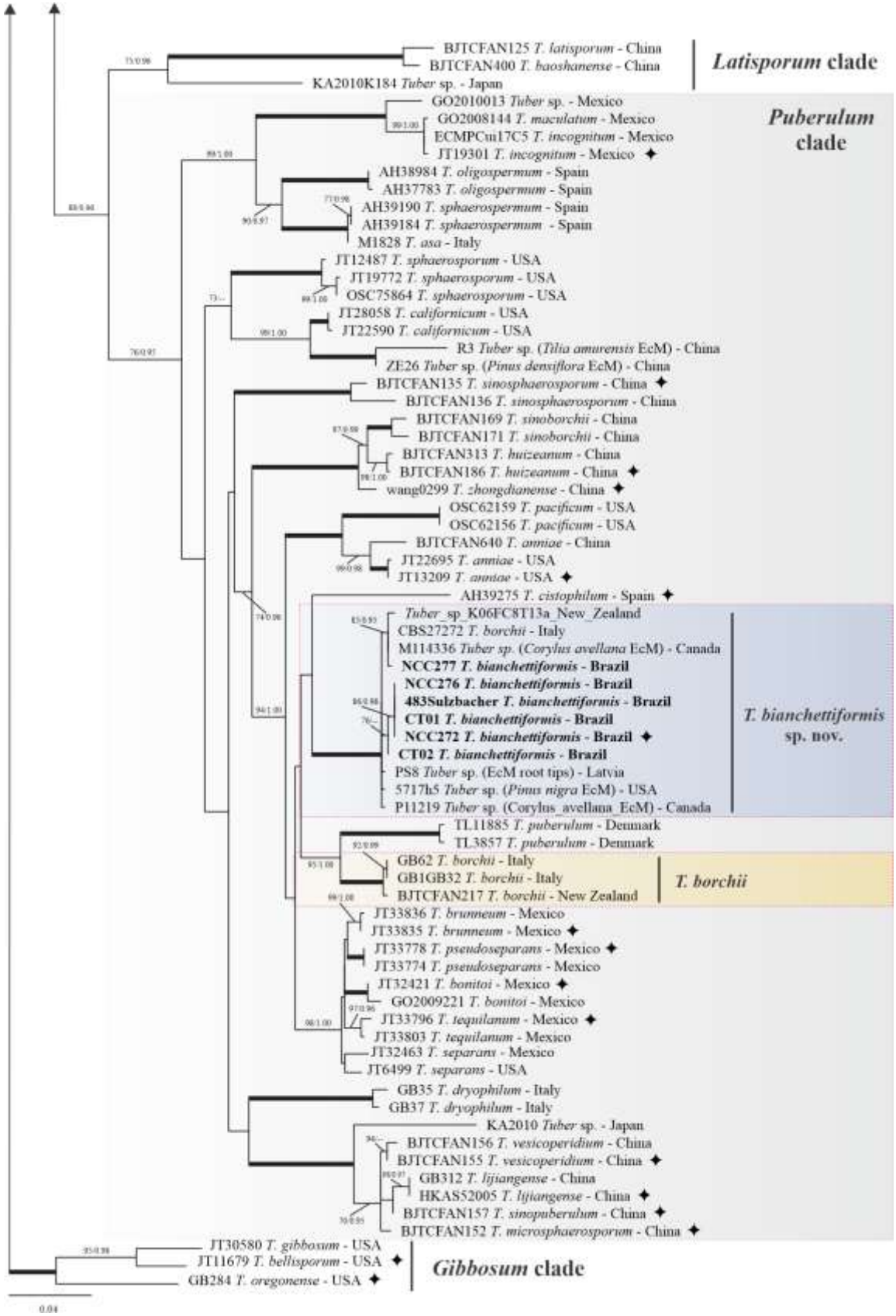

Figure 1. ML phylogeny of representatives of the *Maculatum* and *Puberulum* clades and closely related taxa inferred from ITS sequences (dataset I). Branches are annotated when supported by ≥70% bootstrap (BS; left) or ≥0.95 Bayesian posterior probability (BPP; right). Thick branches indicate maximum support (BS = 100; BPP = 1.00). Collections sequenced in the present study are shown in boldface. Black stars indicate holotype specimens.

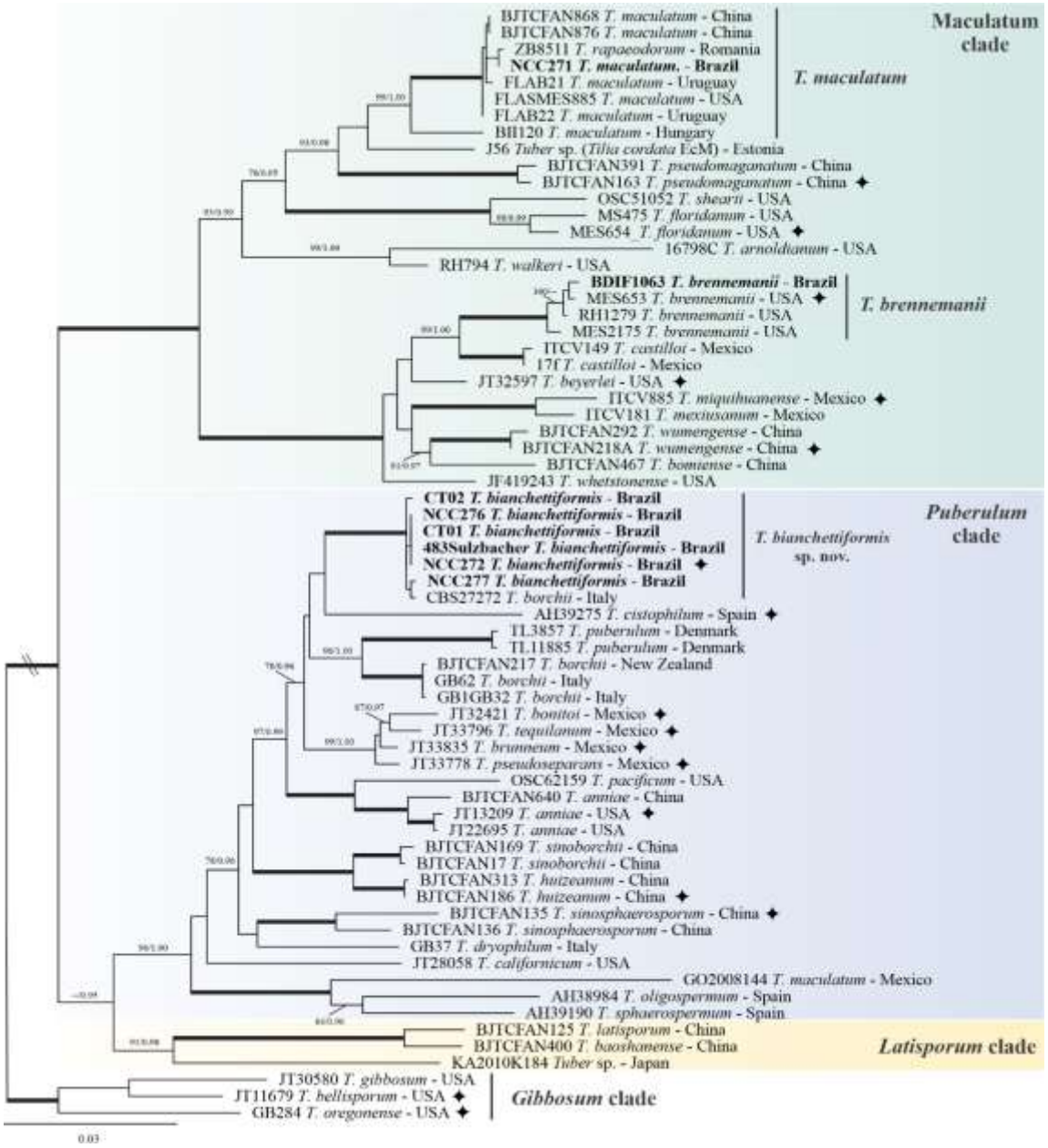

Figure 2. ML phylogeny of representatives of the *Maculatum* and *Puberulum* clades and closely related taxa inferred from combined analyses of ITS+nrLSU+*rpb2*+*tef1-α* sequences (dataset II). Branches are annotated when supported by ≥70% bootstrap (BS; left) or ≥0.95 Bayesian posterior probability (BPP; right). Thick branches indicate maximum support (BS = 100; BPP = 1.00). Collections sequenced in the present study are shown in boldface. Black stars indicate holotype specimens.

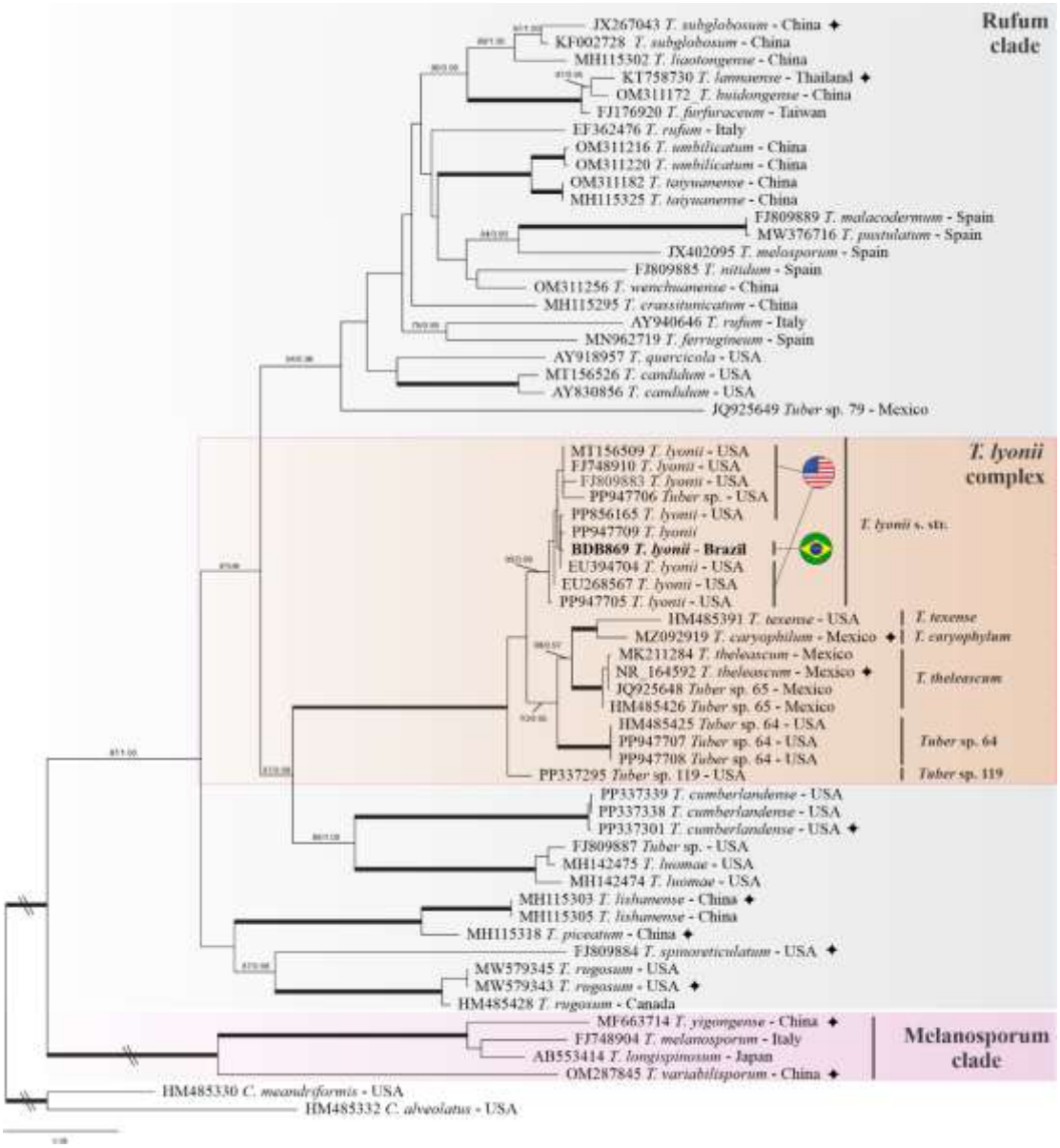

Figure 3. ML phylogeny of representatives of the *Rufum* clade and closely related taxa inferred from ITS sequences (dataset III). Branches are annotated when supported by ≥70% bootstrap (BS; left) or ≥0.95 Bayesian posterior probability (BPP; right). Thick branches indicate maximum support (BS = 100; BPP = 1.00). Collections sequenced in the present study are shown in boldface. Black stars indicate holotype specimens.

Within the Puberulum clade, both single-locus (dataset I) and concatenated multilocus phylogenetic analyses (dataset I and II) consistently supported the distinct phylogenetic placement of the Brazilian specimens herein identified and described as *Tuber bianchettiformis*, distinguishing them from closely related *Tuber* taxa (Figures 1, 2). *Tuber bianchettiformis* formed a well-delimited monophyletic lineage that united the Brazilian specimens sequenced in this study with conspecific accessions from Europe, North America, and New Zealand, receiving maximal support (BS = 100%; BPP = 1.00) in both ITS and combined analyses (Figures 1, 2). *Tuber cistophilum* P. Alvarado, G. Moreno, Manjón, Gelpi & Jaime Muñoz, originally described from Spain, is unsupported sister to *T. bianchettiformis*. Moreover, the

clade encompassing *T. bianchettiformis* and *T. cistophilum* was inferred in both ITS and combined analyses (Figures 1, 2) as unsupported sister to a strongly supported lineage (BS = 95–96%; BPP = 1.00) that includes *T. puberulum* Berk. & Broome from Denmark and *T. borchii* from Italy and New Zealand.

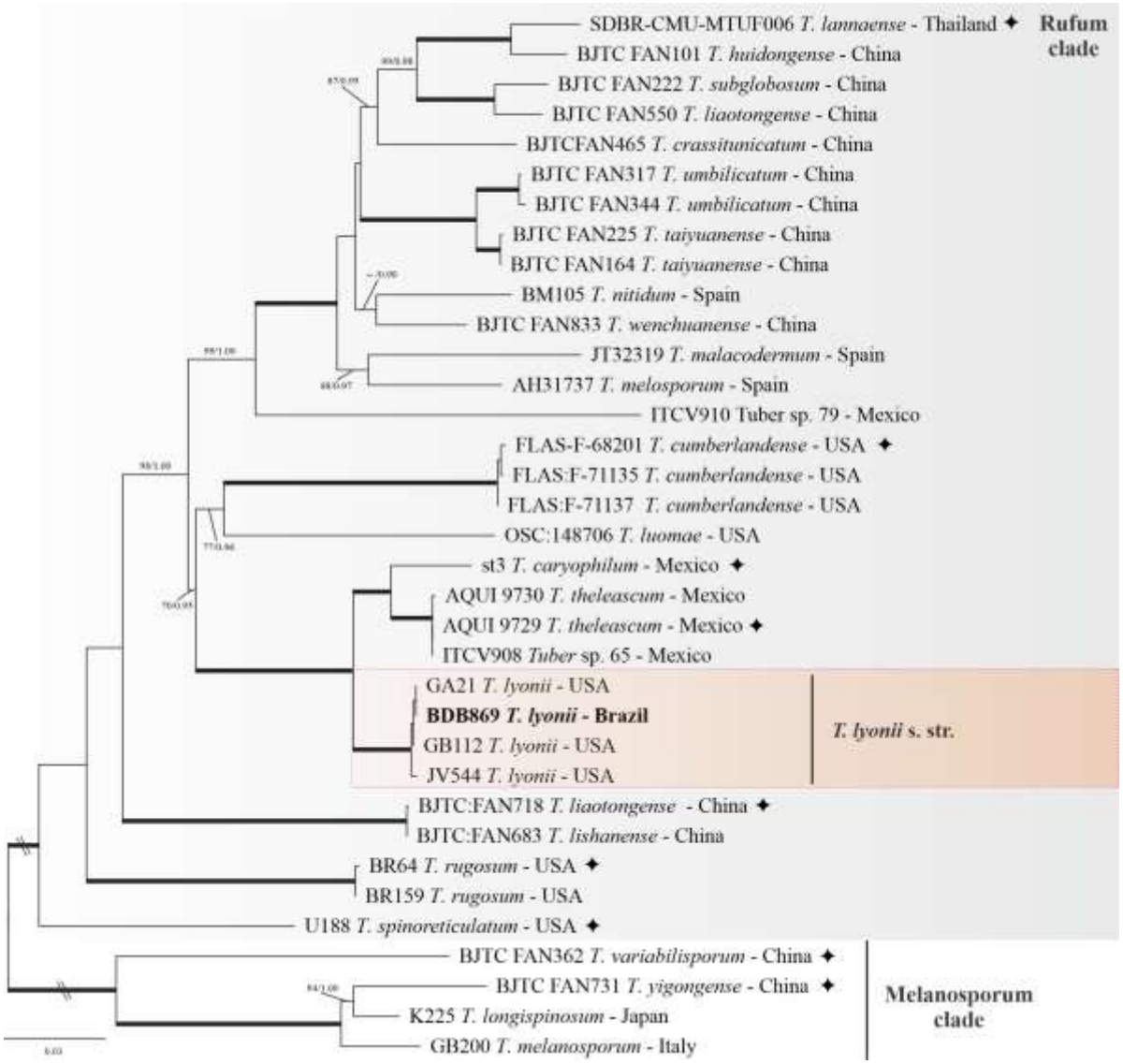

Figure 4. ML phylogeny of representatives of the *Rufum* clade and closely related taxa inferred from combined analyses of ITS+nrLSU+*rpb2*+*tef1-α* sequences (dataset IV). Branches are annotated when supported by ≥70% bootstrap (BS; left) or ≥0.95 Bayesian posterior probability (BPP; right). Thick branches indicate maximum support (BS = 100; BPP = 1.00). Collections sequenced in the present study are shown in boldface. Black stars indicate holotype specimens.

TAXONOMY

Tuber bianchettiformis Coelho-Nascimento & Sulzbacher, **sp. nov.** Figure 5.

MycoBank MB000000

Typification: SÃO PAULO: São Bento do Sapucaí, Entre Vilas, Serra da Mantiqueira, developing hypogeously in association with *Corylus avellana* and *Pinus* sp., 11 May 2023, *R.I. Veraldi* NCC272 (FIFUNGI 1175), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

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Diagnosis: The characteristics that differentiate *Tuber bianchettiformis* from the closely related *T. borchii* include its smaller ascomata (up to 30 mm in diameter), smaller spores (32–47 × 20–39 µm) that are broadly ellipsoid to ellipsoid with fewer alveolae (4–8 lengthwise), and a narrower peridial layer with cell walls not exceeding 2 µm in thickness. This new species is also clearly differentiated from other closely related species in ITS and multilocus phylogenies.

Etymology: Referring to its morphological resemblance to *Tuber borchii*, the well-known Bianchetto truffle.

Ascoma 8–30 mm diam., hypogeous, subglobose to irregularly lobed, occasionally gibbous or with a flattened base lacking basal excavation; surface dry, initially finely pubescent, becoming glabrous or nearly smooth at maturity, at first yellowish white (4A2) to light ochre (5C4), becoming predominantly pale orange-brown (6C4) to ochraceous (5D4), sometimes exhibiting subtle mottling with yellowish gray (4B2), light brown (6D4), and cinnamon tones (6D5–7C4), particularly along shallow furrows, lobes, and near the base. **Gleba** solid, firm, whitish (4A2) when young, becoming beige (4B3–4C4) to ochraceous with reddish hues (5C4–6C4), then greyish brown (6D4) to reddish brown (7E4) at maturity; marbled with white (1A1) to pale cream (4A2), wide, anastomosing veins that originate from various points on the peridium. **Odor** distinctly garlicky with nutty nuances, developing a stronger sulfurous component in overmature ascomata. **Taste** not recorded.

Peridium undetachable, 180–300 µm thick, two-layered, external layer (ectal excipulum) 90–150 µm thick, pseudoparenchymatous, composed of subglobose, irregularly ellipsoid to polygonal cells 10.0–40 µm diam., pale ochraceous brown on the surface, subtended by hyaline and thick-walled (up to 2.0 µm thick) cells,; dermatocystidia 12.0–40 × 2.5–5.5 µm at the base, scattered, narrowing gradually toward the apex, one- to three-celled, moderately thick-walled, hyaline in KOH; internal layer (medullary excipulum) 50–130 µm thick, prosenchymatous, composed of loosely interwoven, mainly periclineal hyaline hyphae 3.0–8.0 µm diam., thin- to slightly thick-walled, intermixed with occasional isodiametric cells. Gleba hyphae 4.0–12.0 µm diam., forming a continuum with the medullary excipulum, without marked structural differentiation.

Asci 65–103 × 45–72 µm, broadly ellipsoid to ellipsoid, sometimes elongate, hyaline, thick-walled, sessile, containing 1–4 ascospores.

Ascospores pale golden brown in KOH, broadly ellipsoid to ellipsoid, rarely elongate, regularly alveolate with mostly hexagonal but sometimes pentagonal, rectangular or irregular alveolae numbering 4–8 lengthwise and 3–6 widthwise; alveolae 7.0–10.0 µm wide, 3.0–5.5 µm high. Ascospores from 1-spored asci 38–47 × 30–39 µm, Q = 1.21–1.88 (Qm = 1.32 ± 0.08); in 2-spored asci 34–38 × 26–32 µm, Q = 1.18–1.46 (Qm = 1.27 ± 0.05); in 3- and 4-spored asci 28–32 × 20–27 µm, Q = 1.15–1.42 (Qm = 1.25 ± 0.07). Species naturally present in all Europe

Distribution and ecology: A species naturally occurring across several regions of Europe. This biogeographic signal is corroborated by comparative analyses of ITS sequences, which demonstrate 99–100% pairwise identity between Brazilian collections and newly curated European accessions (data not shown). These reference sequences were kindly provided by the co-author Tine Grebenc, although they have not yet been formally included in this version of the manuscript due to pending authorship arrangements (Grebenc “com. pess.” 2025). Comparative analyses of ITS sequences available in GenBank and those generated in this study, including sequences from ectomycorrhizal root tips, suggest that *Tuber bianchettiformis* has also been introduced to North America, South America, and Oceania., most likely via inadvertent transport of non-inoculated *Pinus nigra* and *Corylus avellana* saplings carrying dormant propagules in root-associated soil.

Specimens examined: BRAZIL. MINAS GERAIS, Camanducaia, Serra da Mantiqueira, Sítio Mandaçaia, hypogeous in soil associated with *Corylus avellana*, 24 May 2022, *C. Coelho-Nascimento & T.V.D.H. Comenale CT01* (FIFUNGI 1179), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000; *ibid. CT02* (FIFUNGI 1180), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000. SÃO PAULO: São Bento do Sapucaí, Entre Vilas, Serra da Mantiqueira, developing hypogeously in association with *Corylus avellana* and *Pinus* sp., 11 May 2023, *R.I. Veraldi NCC272* (holotype, FIFUNGI 1175); *ibid. NCC276* (FIFUNGI 1176), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid. NCC277* (FIFUNGI 1177), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000; *ibid.* 02 Jul. 2022, *M. A. Sulzbacher & R.I. Veraldi 483-Sulzbacher* (FIFUNGI 1178), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Notes: *Tuber bianchettiformis* belongs to the Puberulum clade. Among the species included in this group, *T. bianchettiformis* has the most in common with *T. borchii*, a species naturally present in all Europe, with similar characters that include: a whitish to brownish ascoma with a

reddish brown gleba, a pseudoparenchymatous peridium, sessile asci containing 1–4 ascospores, with reticulate-alveolate spores having a rather wide range of Q, etc (Leonardi *at al*. 2021b). However, *T. borchii* has stouter ascomata, measuring up to 70 mm diam.; its ascospores with Q values between 1.06 and 1.46 and bear a reticulum with more alveolae (4–11 lengthwise); and its peridial layer is distinctly broader (150–600 µm thick), with collenchymatic-type cell walls up to 4.0 µm thick (Leonardi *at al*. 2021b), whereas that of *T. bianchettiformis*, 180–300 µm thick, has more regular walls not exceeding 2.0 µm in thickness.

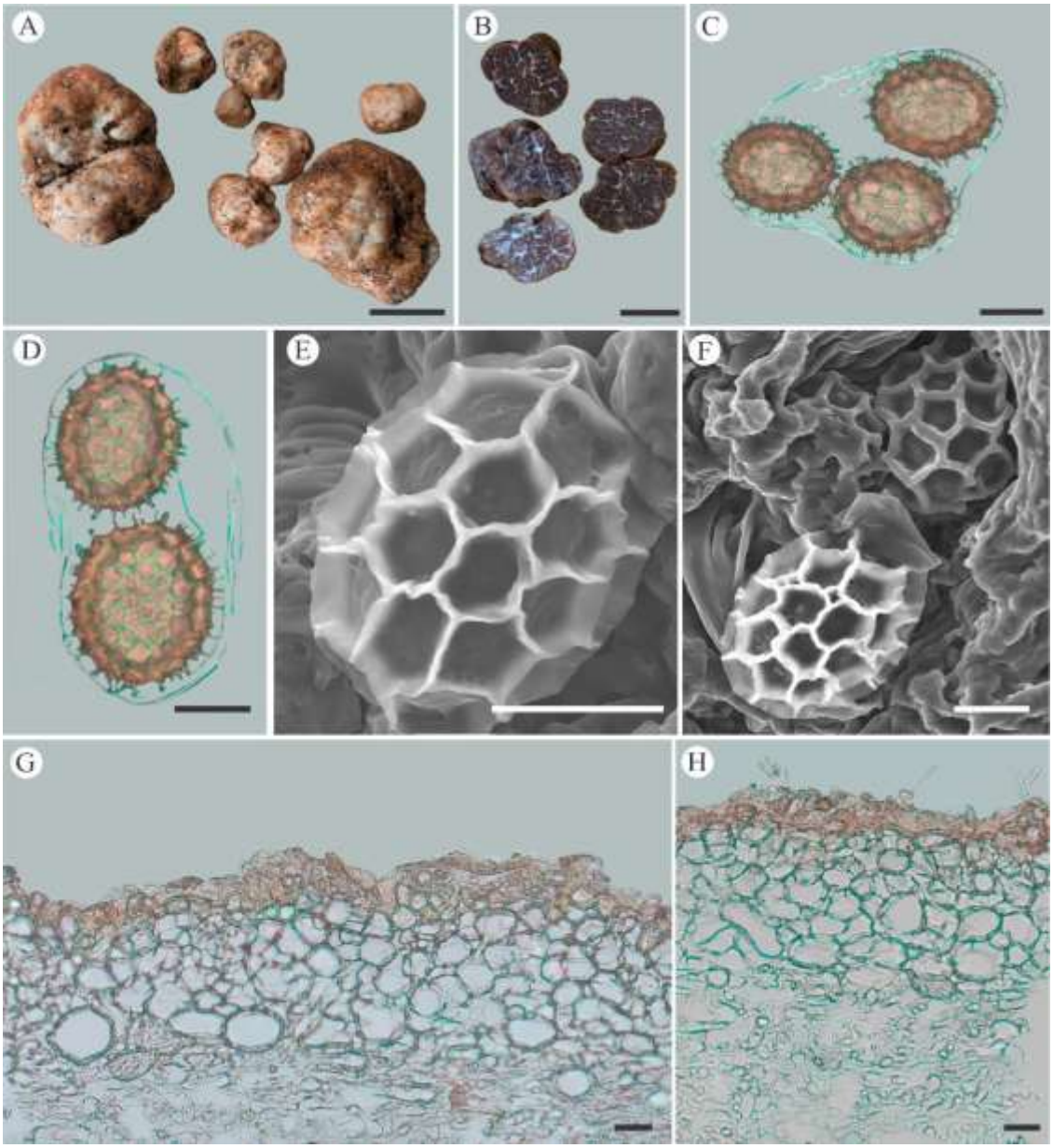

Figure 5. *Tuber bianchettiformis* (NCC272, FIFUNGI 1175, holotype). **A**. Ascomata. **B**. Gleba detail. **C**–**D**. Ascus with ascospores. **E**–**F**. Ascospore details featuring ornamentation of the polygonal meshes. **G**. Peridium external layer structure (ectal excipulum). **G**. Peridium showing bilayer structure. Bars: A–B = 10 mm; C–H = 20 µm.

Of the “white truffles” taxa in Puberulum group originally described from Europe, the

the following must be considered with regard to our claim of novelty for *T. bianchettiformis*: *Tuber cistophilum*, *Tuber oligospermum* (Tul. & C. Tul.) Trappe, and *Tuber puberulum*.

Tuber cistophilum, from southwestern Spain, is different from *T. bianchettiformis*, by the orange-brown to ochraceous ascoma with dark gleba when mature, a prosenchymatous peridium, and a weak odor (Alvarado et al. 2012). In addition, *T. cistophilum* is known to occur exclusively in association with *Cistus ladanifer* L. (Alvarado et al. 2012).

Tuber oligospermum, from France (Tulasne & Tulasne 1851), has ascomata of similar size and color to *T. bianchettiformis*, but it can be distinguished by a number of characters, including a prosenchymatous peridium, the subglobose asci with short pedicels, the generally subglobose ascospores, and a faint odor (Alvarado et al. 2012).

Tuber puberulum, from southern England (Berkeley & Broome 1846), resembles *T. bianchettiformis* in its whitish ascoma and pseudoparenchymatous peridium with large cells, but deviates from the latter in its paler gleba, the generally subglobose ascospores, and a faint raphanoid odor (Pegler et al. 1995, Alvarado et al. 2012). *Tuber puberulum* is reported to be associated with conifers or broad-leaved trees (e.g. *Quercus ilex* L., *Populus* sp., and *Pinus halepensis* Mill.) (Pegler et al. 1995, Alvarado et al. 2012).

Tuber brennemanii A.C. Grupe, Healy & M.E. Sm., Mycologia 110(4): 780 (2018) Figure 6.

Ascoma 18–38 mm diam., hypogeous, knotty and irregularly lobed or subglobose, pale yellowish brown (5C4, 5D4) to ochraceous brown (6D5, 6E5); surface undetachable, smooth to minutely verrucose or areolate in some spots, with few lighter-colored furrows. **Gleba** firm, solid, brownish grey (5E3, 5F4) to umber (6F3) at maturity, slightly darkening with age or desiccation, marbled with white (1A1) to off-white veins. **Odor** pleasant. **Taste** nutty.

Peridium undetachable composed of two layers; an external layer (ectal excipulum) 85–274 µm thick, pseudoparenchymatous, composed of globose to subglobose, angular or oblong cells approximately 6.0–29 µm diam., thick-walled (up to 1.5 µm thick), hyaline to yellow-brown or reddish brown; with an internal layer (medullary excipulum) 35–140 µm thick composed of periclinal to tightly interwoven hyphae 2.5–6.0 µm diam., hyaline, thin to slightly thick-walled. **Glebal hyphae** 2.0–6.0 µm diam, hyaline or yellow-brown in mass, thin-walled, sometimes inflated.

Asci 55–98 × 38–80 µm, globose, irregularly subglobose to ellipsoid or rarely oblong, usually without but rarely with a short pedicel measuring 4.0–6.0 × 2.0–3.0 µm, hyaline in KOH, hyaline to reddish brown with purplish walls in Melzer's reagent, thick-walled (up to 3.0 µm thick), formed by two visible layers in KOH, with 1–4 ascospores per ascus.

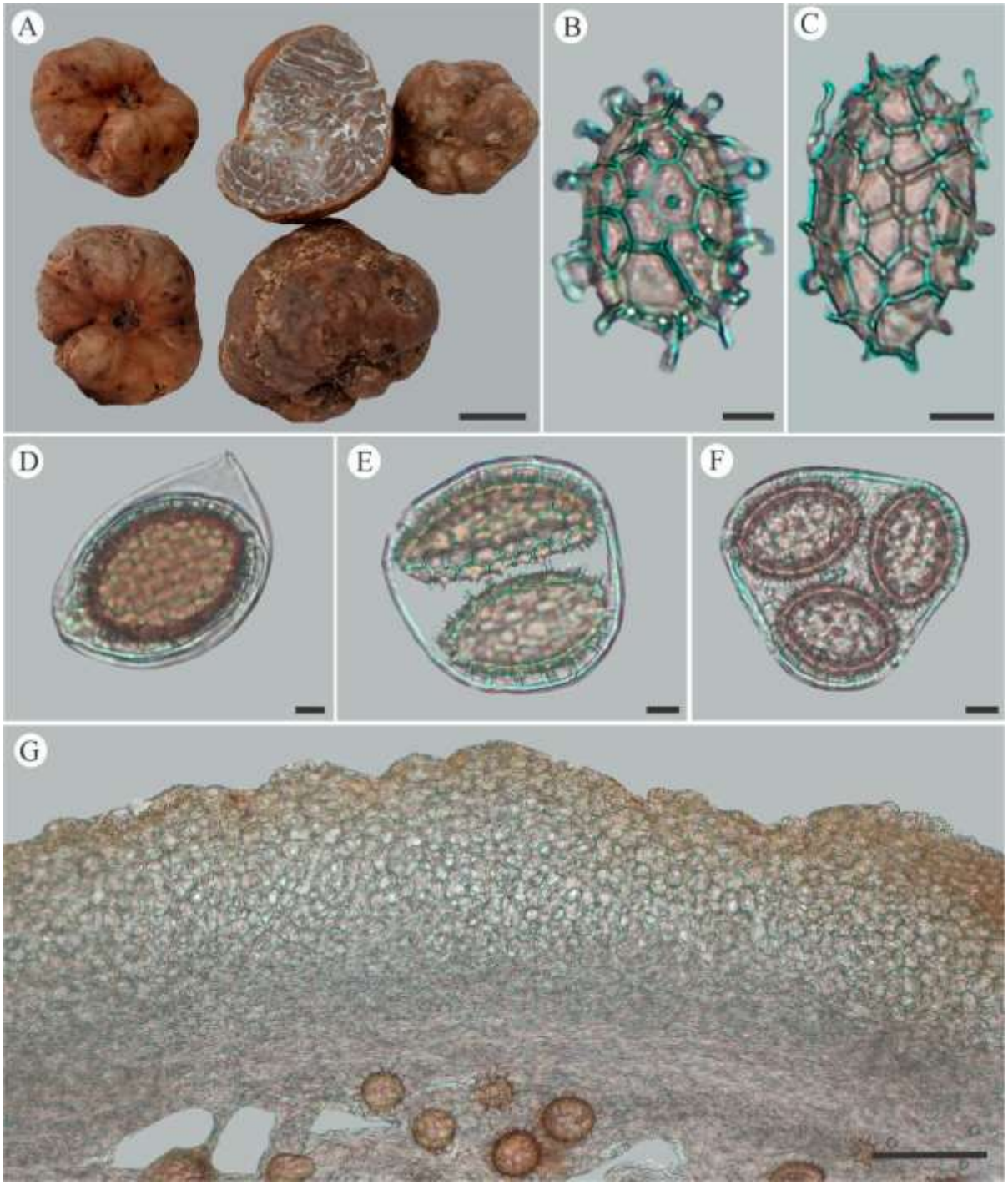

Figure 6. *Tuber brennemanii* (BDIF1060, FIFUNGI 1181). **A**. Ascomata and gleba. **B**–**C**. Ascospore details featuring ornamentation of the polygonal meshes. **D**–**F.** Ascus with ascospores. **G**. Peridium external layer structure (ectal excipulum). **G**. Peridium showing bilayer structure. Bars: A = 10 mm; B–F = 10 µm; G = 100 µm.

Ascospores reddish brown to yellow-brown, ellipsoid to broadly ellipsoid, rarely elongate or cylindrical, reticulate–alveolate; reticulum with 4–10 meshes lengthwise and 4–8 widthwise, the alveolar walls 4.0–8.0 µm tall, some ascospores subalveolate or with irregular reticulation. Ascospores from 1-spored asci 30–62 × 19.5–42 µm, Q = 1.34–1.89 (Qm = 1.5 ± 0.09); in 2-

spored asci 30–57 × 19.0–34 µm, Q = 1.38–2.2 (Qm = 1.55 ± 0.15); in 3-spored asci 25–54 × 15.0–32 µm, Q = 1.32–2.00 (Qm = 1.42 ± 0.08); in 4-spored asci 23–42 × 13.0–30 µm, Q = 1.24–1.98 (Qm = 1.40 ± 0.14). **Anamorph colonies** not observed.

Distribution and ecology: It occurs across eastern North America, from Massachusetts to Georgia, westward to Minnesota and Texas, and south to Nuevo León in northern Mexico (Grupe II et al. 2018). Although it occurs in native forests, it is more frequently found in disturbed environments such as pecan orchards and residential woodlands (Grupe II et al. 2018). It forms ectomycorrhizal associations primarily with *Carya illinoinensis*, *Quercus* spp., and other members of the Fagales (Grupe II et al. 2018). It is reported here for the first time from Brazil, based on a collection from the state of Rio Grande do Sul.

Specimen examined: BRAZIL. RIO GRANDE DO SUL: Cachoeira do Sul, Estrada Botucaraí, RS-403, Km 48, in a commercial pecan (*Carya illinoinensis*) orchard, 25 Nov. 2024, *M.A. Sulzbacher BDIF1063* (FIFUNGI 1181), GenBank [ITS]: 00000000.

Notes: *Tuber brennemanii* was originally described from Georgia, USA, and had previously been recorded only from North America, including northern Mexico (Grupe II et al. 2018). In Brazil, it was found in the state of Rio Grande do Sul, sharing the same ectomycorrhizal host association as *Tuber floridanum* at the site.

Tuber lyonii Butters, Bot. Gaz. 35 (6): 431 (1903) Figure 7.

Ascoma 16–52 mm diam., hypogeous, subglobose to irregularly lobed, often with shallow to deep furrows; surface bald, dry, smooth to slightly roughened, orangish brown (6–7E4) to yellowish tan (5C4, 5D4), occasionally dark brown (8F4–5) with maturity. **Gleba** solid, firm, watery grayish when young, becoming light brown (6D4, 6E4) to brownish orange (6C4) with age, and dark umber (7F8) upon desiccation; consistently marbled with fine, white (1A1) to cream (4A2–3) veins radiating from the base. **Odor** pungent, truffle-like, slightly musty and nutty. **Taste** not recorded.

Peridium undetachable, composed of two layers: a distinct thinner external layer (ectal excipulum), 40–60 µm thick, pseudoparenchymatous, composed of pale orange-brown, straw-yellow, and hyaline globose, subglobose, polygonal, or irregular cells, approximately 5.0–17.0 µm in diam., thick-walled (up to 2.5 µm thick); and an internal layer (medullary excipulum), 232–280 µm thick, pseudoparenchymatous composed of strongly interwoven hyphae, 2.0–5.0 µm diam. at septa, hyaline, thin- to slightly thick-walled. **Glebal hyphae** 3.0–7.7 µm diam., hyaline, thin-walled, periclinally arranged, sometimes slightly inflated.

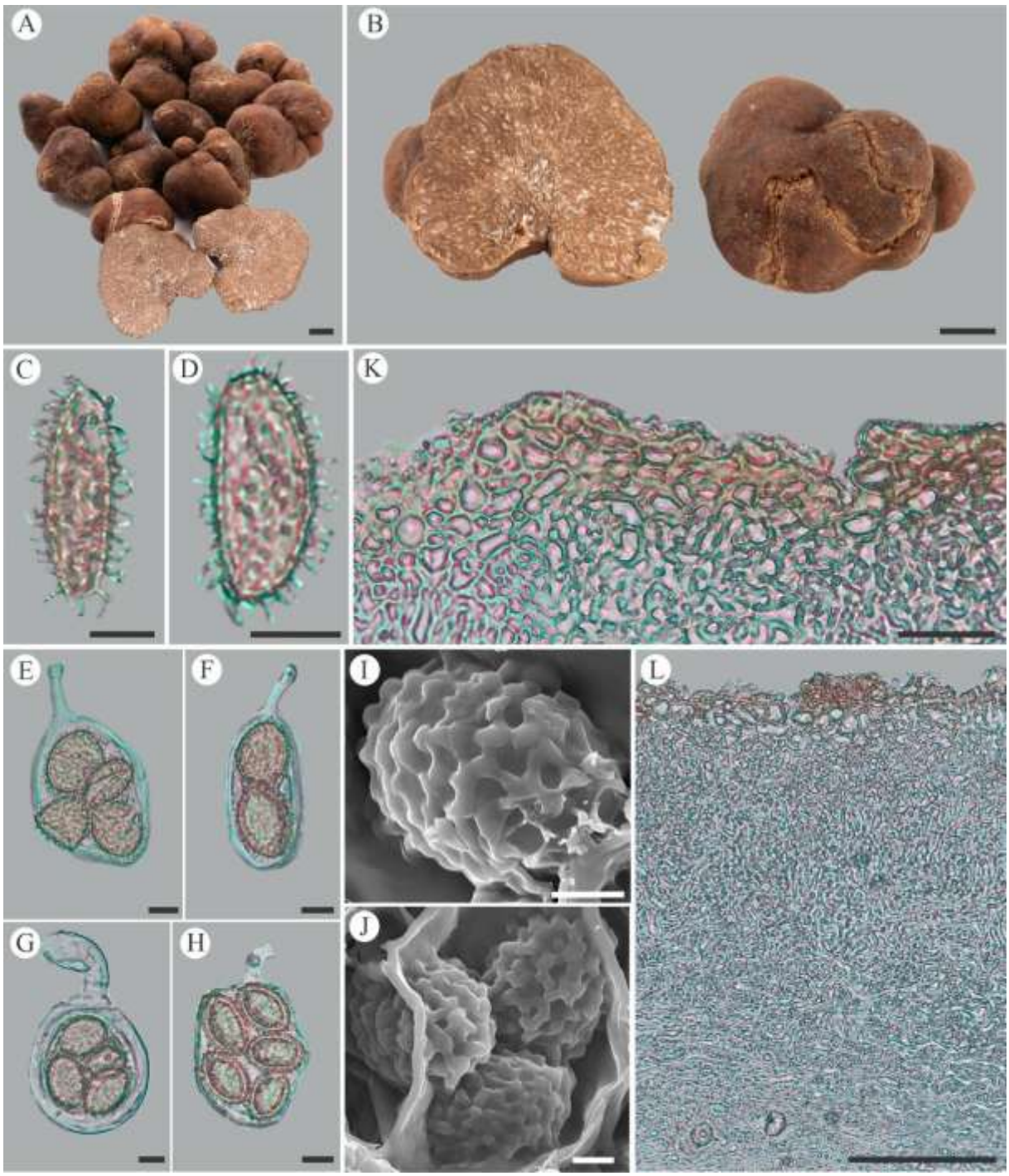

Figure 7. *Tuber lyonii* (BDB869, FIFUNGI 1182). **A**–**B**. Ascomata and gleba. **C**–**D**. Ascospores. **E**–**H.** Ascus with ascospores. **I–J**. Ascospore details featuring ornamentation. **K**. Peridium external layer structure (ectal excipulum). **L**. Peridium showing bilayer structure. Bars: A–B = 10 mm; C–H, K = 20 µm; I–J = 5 µm ; L = 100 µm.

Asci 70–117 × 50–70 µm including a long, stout, bifurcated pedicel measuring 31–44 × 9.2–14.5 µm, from subglobose to ellipsoid, rarely elongate, hyaline, formed by three visible layers in KOH, cell wall up to 5 µm thick, containing 1–5 ascospores per ascus.

Ascospores yellowish to pale reddish brown in KOH, mostly ellipsoid, but also elongate to cylindrical, spinose-reticulated; spines straight 3.5–7.0 µm high, united at the base by a ridged network, forming small alveola with 4–6 sides. Ascospores from 1-spored asci 44–67 × 26–33

µm, Q = 1.47–2.17 (Qm = 1.70 ± 0.15); in 2-spored asci 38–58 × 24–32 µm, Q = 1.49–1.93 (Qm = 1.60 ± 0.05); in 3-spored asci 39–50 × 23–28 µm, Q = 1.50–1.83 (Qm = 1.58 ± 0.12); in 4-spored asci 36–44 × 22–26 µm, Q = 1.47–1.89 (Qm = 1.55 ± 0.14); in 5-spored asci 34–41 × 21–25 µm, Q = 1.44–1.83 (Qm = 1.56 ± 0.9)

Distribution and ecology: It occurs from northern Mexico to the Canadian province of Québec, and from the state of New Mexico (USA) to the eastern seaboard (Trappe et al. 1996; Smith et al. 2012), primarily in association with *Carya illinoinensis* but also with a wide variety of angiosperm hosts, including species of *Betula*, *Quercus*, and other Fagales (Coleman et al. 2024). It is found in native forests, orchards, and residential areas. Most reports come from the states of Georgia and Florida, in the USA (Grupe II et al. 2016). It is reported here for the first time from Brazil, based on a collection from the state of Rio Grande do Sul.

Specimen examined: BRAZIL. RIO GRANDE DO SUL: Cachoeira do Sul, Estrada Botucaraí, RS-403, Km 48, in a commercial pecan (*Carya illinoinensis*) orchard, 20 Mar. 2024, *M.A. Sulzbacher BDB869* (FIFUNGI 1182) GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000.

Notes: *Tuber lyonii* is a spiny-spored truffle species native to North America and originally described by Butters (1903) based on material collected in the state of Minnesota. Commonly referred to as the "pecan truffle," *T. lyonii* has garnered increasing interest due to its frequent ectomycorrhizal association with *Carya illinoinensis* (pecan) across various regions of the southeastern and midwestern USA (Trappe et al. 1996; Smith et al. 2012). Although long regarded as a single, broadly distributed species throughout eastern North America, recent molecular studies have revealed that *T. lyonii*, in its traditional sense (Butters 1903, Trappe et al. 1996), actually represents a complex of closely related but genetically distinct lineages (Leonardi et al. 2019; Sanchez-Ledesma et al. 2022, Rennick et al. 2023).

In the present study, we report the occurrence of *T. lyonii* in Brazil for the first time, based on a single specimen (BDB869) collected from commercial *Carya illinoinensis* (pecan) orchards in the state of Rio Grande do Sul. The specimen exhibits complete morphoanatomical congruence with the Butters' protologue of *T. lyonii* (Butters 1903) and is here interpreted as representing *T. lyonii* s. str. It is also worth mentioning that the taxonomic identity of *T. texense*, a species morphologically similar to *T. lyonii*, remains unresolved. It was represented in the ITS-based phylogeny by a singleton sequence (Figure 3), *T. texense* may ultimately prove to be a later synonym of *T. lyonii* s. str. Alternatively, the sequence currently attributed to *T. texense* (HM485391) may correspond to an undescribed species within the *T. lyonii* complex, underscoring the need for further taxonomic investigation.

The discovery of *T. lyonii* in Brazil significantly expands the known distributional range of the species and raises important questions regarding its biogeographic origin in South America. Although natural dispersal cannot be entirely ruled out, the most plausible hypothesis is that this lineage was introduced in South America via pecan saplings imported from North America, potentially pre-inoculated or carrying dormant propagules in root-associated soils. Naturalization of *T. lyonii* in managed orchards has already been reported in North America, where it forms stable ectomycorrhizal associations and may persist independently from inoculation efforts (Coleman et al. 2024).

Alongside *T. canaliculatum* Gilkey and species of the complex involving *T. gibbosum* Harkn., *T. lyonii* is one of the few North American truffles with established commercial appeal, being actively harvested and marketed in the USA (Coleman et al. 2024). The occurrence of *T. lyonii* in Brazilian pecan orchards presents new opportunities for research and potential cultivation in South America.

Tuber maculatum (Pers.) Jülich, Bibliotheca mycologica 85: 399 (1982) Figure 8.
= *Tuber maculatum* var. *ferraresei* G. Gross, *Z. Mykol.* 62(2): 178 (1996)

Ascoma 15–40 mm diam., hypogeous, knotty and irregularly lobed or subglobose, at first yellowish white (4A2) or orange white (5A2), then light tan (4B3-4) to yellow tan (5B3,6B2) or beige (4B3, 4C3) to pale yellow-brown (4C4-6), sometimes with reddish or purplish hues, changing from greyish red (8C3-4) to brownish red (8C5-6) when bruised, darkening with maturity; surface hygrophanous, undetachable, smooth to finely granulose, with few superficial furrows. **Gleba** firm, solid, and whitish at first, becoming pale brown (6C2, 7C2) to pale orange brown (5C3) or beige (4B3, 4C3) at maturity, darkening with age or desiccation, marbled with white (1A1) to cream (4A3) veins. **Odor** pungent, raphanoid (radish-like). **Taste** mild to slightly nutty or earthy.

Peridium hygrophanous, undetachable, mostly prosenchymatous, thicker at rugose folds, composed of two layers: a thin external layer (ectal excipulum) 50–100 µm thick, composed of versiform, angular or subglobose hyphae approximately 8.5–25 µm diam., thick-walled, buff-yellow to cream or hyaline; and a well delimited internal layer (medullary excipulum) 130–238 µm thick, composed of prostrate and loosely interwoven hyaline hyphae 6.0–9.0 µm diam., hyaline, with the basal part of internal layer gradually intermixing into the gleba. **Glebal hyphae** hyaline, thin-walled, cylindrical to subinflated (6.0–17.0 µm diam.) cells, intermixed with subglobose to clavate cells (8.0–19.5 µm diam.).

Asci 45–93 × 42–72 µm, subglobose to ellipsoid or pyriform, usually without but rarely with a short pedicel measuring 3.0–8.0 × 1.5–2.5 µm, hyaline, thin-walled, formed by two visible layers in KOH, with 1–4 ascospores per ascus, mostly 3- and 4-spored asci.

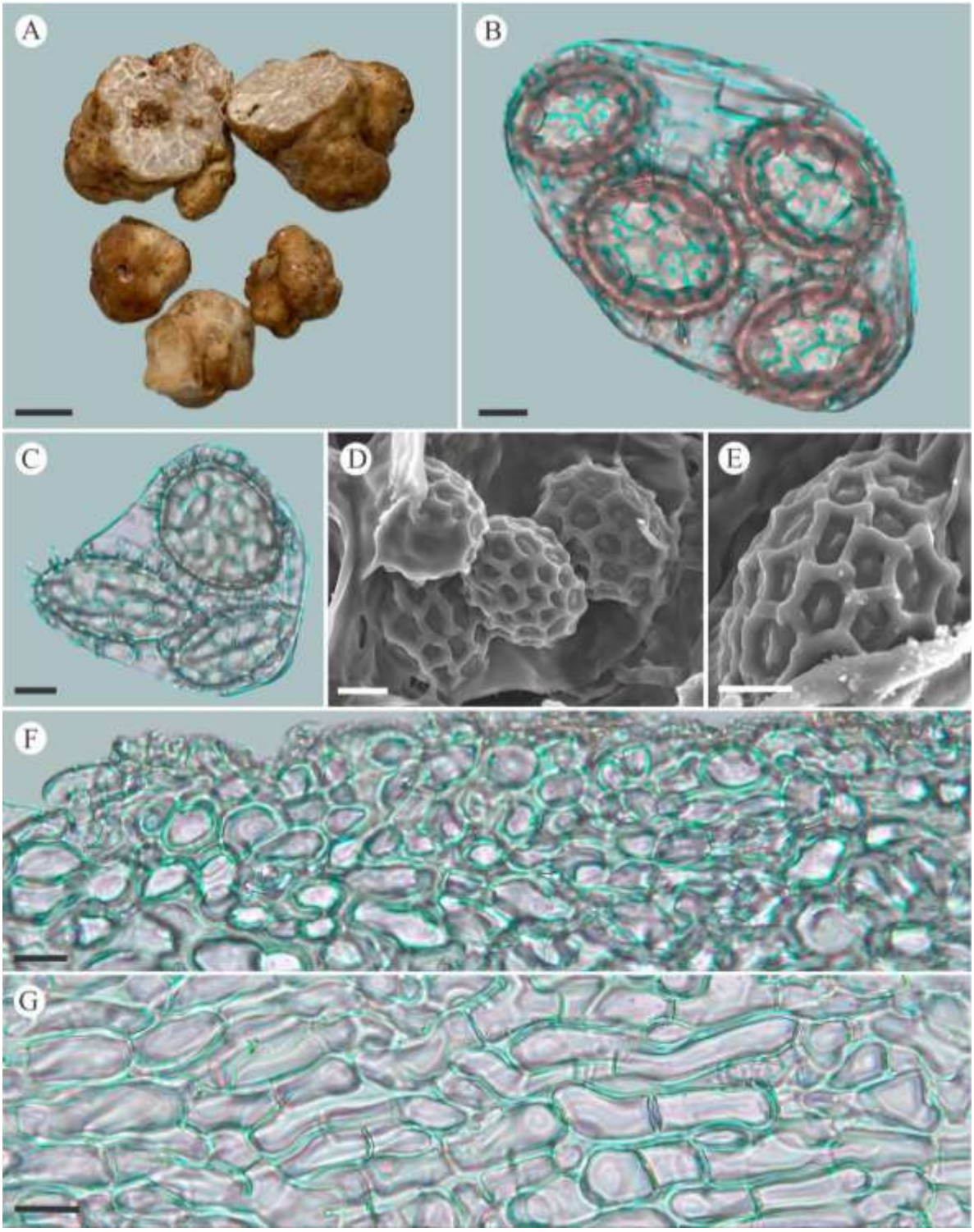

Figure 8. *Tuber maculatum* (NCC271, FIFUNGI 1183). **A**. Ascomata and gleba. **B**–**C**. Ascus with ascospores. **D**–**E**. Ascospore details featuring ornamentation of the polygonal meshes. **F**. peridium external layer structure (ectal excipulum). **G**. peridium internal layer structure (medullary excipulum). Bars: A = 10 mm; B–G = 10 µm.

Ascospores pale golden yellow (4A4–5) to pale yellowish brown (4–5D4), broadly ellipsoid to ellipsoid, rarely elongate, reticulate–alveolate, with broad meshes, 2.0–4.0 µm in height; meshes regular, closed, and regularly polygonal (5–6 sides), 4.5–8.0 lengthwise, creating the illusion of spiny ornamentation when observed under light microscopes. Ascospores from 1-spored asci 45–62 × 35–47 µm, Q = 1.20–1.78 (Qm = 1.45 ± 0.1); in 2-spored asci 36–45 × 26–38 µm, Q = 1.30–1.90 (Qm = 1.50 ± 0.08); in 3-spored asci 20–42 × 20–30 µm, Q = 1.20–1.58 (Qm = 1.40 ± 0.12); in 4-spored asci 29–44 × 20–30 µm, Q = 1.25–1.68 (Qm = 1.40 ± 0.18).

Distribution and ecology: Tuber maculatum is among the most widespread European native truffle species and has been introduced to Asia, the Americas, and Oceania via *Quercus* L. seedling (Iotti et al. 2002, Ławrynowicz 2009, Mello et al. 2000, Marjanović et al. 2010, Ori et al. 2023, Wilgan et al. 2023). It is primarily associated with broadleaf deciduous trees, including *Quercus* spp., *Fagus sylvatica, Corylus avellana*, and *Populus* spp. (Mello et al. 2000, Marjanović et al. 2010, Wilgan et al. 2023). In South America, the species has been recorded associated with exotic trees, including *Betula pendula* Roth, *Carya illinoinensis*, and *Pinus contorta*, in Argentina and Uruguay (Lorenzo & Calvelo 2000, Romero & Blumenfield 2001, Kuhar et al. 2024).

Specimen examined: BRAZIL. SÃO PAULO: São Bento do Sapucaí, Entre Vilas, Serra da Mantiqueira, hypogeous in soil near *Betula* sp. and *Quercus* sp., 11 May 2023, *R.I. Veraldi NCC271* (FIFUNGI 1183), GenBank [ITS]: 00000000, [nrLSU]: 00000000, [*rpb2*]: 00000000, [*tef1-α*]: 00000000.

Notes: *Tuber maculatum* is reported here for the first time in Brazil based on specimens collected in a fragment of submontane mixed ombrophilous forest in Southeastern Brazil, where it was found forming ectomycorrhizal associations with species of *Betula* L. and *Quercus*. Such host association reflects the species' ecological flexibility and concurs with earlier records involving Fagaceae and Betulaceae hosts (Mello et al. 2000, Kuhar et al. 2024). Its broad geographic range, combined with low intraspecific genetic divergence, suggests recent range expansion or anthropogenic dispersal (Bonito et al. 2010). This hypothesis is further supported by its recurrent occurrence with non-native trees in Argentina, Uruguay, and now in Brazil (Barroetaveña et al. 2005, 2006, Kuhar et al. 2024). *Tuber maculatum* has also been introduced into managed truffle orchards in Oceania (Trappe & Cázares 2000, Bulman et al. 2010).

The Maculatum clade, long understudied, has proven more diverse than previously assumed, while its species are typically regarded as having limited economic value (Bonito et al. 2010). Nonetheless, *Tuber floridanum*, a member of this group, has been successfully cultivated in southern Brazil and is gaining prominence as a culinary ingredient among local chefs, with growing demand in the supply chains of fine-dining restaurants (Freiberg et al. 2023). This

increasing interest has contributed to the emergence of truffle cultivation initiatives in the country. In a similar vein, *T. maculatum* may also hold potential for at least modest gastronomic and economic relevance, particularly given its morphological and aromatic resemblance to *T. floridanum* (Freiberg et al. 2023; Kuhar et al. 2024). Furthermore, considering its biochemical composition and previously characterized volatile profile, characterized by the predominance of 3-octanone, 3-octanol, and 2H-pyran-2-one, *T. maculatum* represents a promising candidate for applied research in fungal biotechnology (Kuhar et al. 2024).

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Capítulo 5

Taming the giant: first successful cultivation and nutritional composition of *Macrocybe titans* in Brazil

Artigo a ser submetido ao periódico **IMA Fungus**

Taming the giant: first successful cultivation and nutritional composition of *Macrocybe titans* in Brazil

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ABSTRACT

Macrocybe titans is a large edible macrofungus native to the Neotropical region, originally described from Florida. In this study, we report for the first time the successful domestication and cultivation of wild isolates of *M. titans* from Brazil. Six wild isolates grown on PDA were evaluated for in vitro mycelial growth at four temperatures (20, 25, 30, and 35 °C), as well as for spawn production on two cereal grains (wheat and sorghum). Cultivation trials were conducted in polypropylene bags using a compost-based substrate composed of 60% sugarcane bagasse and 40% brachiaria straw (dry weight basis). Mushrooms produced under controlled conditions were analyzed for nutritional and mineral composition. Mycelial growth was optimal at 25–30 °C, and sorghum supported faster colonization than wheat, indicating its suitability for large-scale spawn preparation. Biological efficiency of the isolate MPD643, isolated from Southeastern Brazil, was estimated at 62.23% ± 0.10, following primordia induction under controlled conditions of 24 °C, 85–95% humidity, and a casing layer composed of peat moss,

iron-rich red sand, and vermiculite. Nutritional analysis showed that the produced mushrooms contained 52.9% ± 0.04 carbohydrates, 14.23% ± 0.18 crude fiber, 18.33% ± 0.20 crude protein, 7.57% ± 0.01 crude fat, and 6.97% ± 0.01 ash on dry matter basis. For the mineral content profile, the elements K and P were the most abundant. The species *M. titans* can be cultivated using traditional methods known for other commercial mushroom species and has the potential to be introduced into the edible mushroom industry.

Keywords: Biological efficiency, Callistosporiaceae, edible mushrooms, Mineral content, Mushroom cultivation, Nutritional value.

INTRODUCTION

The genus *Macrocybe* Pegler & Lodge was formally established by Pegler and Lodge in 1998 to accommodate several large-bodied fungi formerly placed in *Tricholoma* (Fr.) Staude and *Agaricus* L (Pegler *et al*., 1998). Originally placed in Tricholomataceae, the genus has undergone subsequent taxonomic reassignments. In a major revision, Vizzini *et al*. (2020) established the family Callistosporiaceae to accommodate *Macrocybe*, grounding this proposal in molecular phylogenetic evidence. Subsequently, Wijayawardene *et al*. (2022) treated the genus as part of Biannulariaceae. More recently, Hyde *et al*. (2024) endorsed the original taxonomic framework proposed by Vizzini and colleagues, retaining *Macrocybe* within Callistosporiaceae, alongside genera such as *Anupama* K.N.A. Raj, K.P.D. Latha & Manim., *Callistosporium* Singer, *Guyanagarika* Sánchez-García, T.W. Henkel & Aime, *Pseudolaccaria* Vizzini, Contu & Z.W. Ge, and *Xerophorus* (Bon) Vizzini, Consiglio & M. Marchetti.

Members of *Macrocybe* are notable for their exceptional size, with some species producing basidiomata with pilei exceeding 100 cm in diameter, and their saprotrophic lifestyle on humus-rich soil (Pegler *et al*., 1998; Karlsen-Ayala & Smith, 2020). Unlike *Tricholoma*, which is characterized by ectomycorrhizal associations and clampless hyphae, *Macrocybe* species possess clamped hyphae and lack symbiotic relationships with plant roots. Comparative genomic studies further support their distinction as a separate lineage (Kui *et al*., 2021). Currently, eight widely accepted species are recognized within the genus, including *M. gigantea* (Massee) Pegler & Lodge, *M. crassa* (Sacc.) Pegler & Lodge, *M. titans* (H.E. Bigelow & Kimbr.) Pegler, *M. lobayensis* (R. Heim) Pegler & Lodge, *M. pachymeres* (Berk. & Broome) Pegler & Lodge, *M. praegrandis* (Berk.) Pegler & Lodge, *M. spectabilis* (Peerally & Sutra) Pegler & Lodge, and *M. sardoa* Vizzini, Consiglio & M. Marchetti (Hyde *et al*., 2024; Peiris *et al*., 2024).

Species of *Macrocybe* are typically distributed across tropical and subtropical regions, occurring in a range of humid habitats such as rainforest floors, forest edges, grasslands, and anthropogenic environments (Pegler *et al*., 1998). These sites are generally characterized by high moisture levels, with average relative humidity around 70%, and warm temperatures ranging from 25 to 28°C (Roy and Krishnappa, 2018).

Macrocybe titans, the type species of the genus and among the largest documented gilled mushrooms, exhibits a geographically widespread presence within Neotropical ecosystems. Originally described from Gainesville, Florida, in 1980, as *Tricholoma titans* H.E. Bigelow & Kimbr. (Bigelow & Kimbrough, 1980), its distribution spans the southeastern United States (Florida, Georgia, Louisiana, North Carolina, South Carolina, and Texas) and Mexico (Bigelow & Kimbrough, 1980; Cifuentes & Guzmán, 1981; Singer, 1990; Lopez & Garcia, 2018), as well as various regions throughout the Caribbean and Central America (Pegler *et al*., 1998; Calonge *et al*., 2007; Piepenbring, 2008; Corrales & López, 2017; Karlsen-Ayala & Smith, 2020). Reports from South America include confirmed occurrences in Argentina, Brazil, Colombia, Ecuador, and Venezuela (Pegler *et al*., 1998; Battistin & Picciola, 2015; Ramírez *et al*., 2017; Chivatá Bedoya, 2018; Luna-Fontalvo *et al*., 2023; Drewinski *et al*., 2024a). The Argentinean collections, reported by Ramírez *et al*. (2017), mark the southernmost record in the Western Hemisphere and blend seamlessly with earlier documented specimens, underscoring a continuous Neotropical distribution. There are also reports of *Macrocybe titans* from India and Taiwan (Vrinda *et al*., 1997; Chen & Chen, 1999; Vrinda & Pradeep, 2006); however, these records are likely misidentifications of *M. crassa*, as suggested by Vizzini *et al*. (2020) based on morphological features and the known biogeographic ranges of both species.

In Brazil, *Macrocybe titans* has been recorded in the Atlantic Forest domain, specifically in the states of Paraná, São Paulo, and Rio Grande do Norte. These occurrences, consolidated by Drewinski *et al*. (2024a), extend the known distribution of the species in the country and include the first publicly available ITS sequences from Brazilian specimens.

Most *Macrocybe* species are edible and exhibit promising agronomic potential, with several already under investigation for cultivation (Teaumroong *et al*., 2002; Verma *et al*., 2017; Galappaththi *et al*., 2022). In addition, they have emerged as valuable sources of nutraceutical and pharmacologically active compounds, exhibiting a wide range of bioactivities relevant to human health (Peiris *et al*., 2024). *Macrocybe gigantea* is one of the most extensively studied species, known for its content of water-soluble polysaccharides, vitamin E, colchicine, and 5-methyldioadenosine, which are associated with antioxidant, antimicrobial, immunomodulatory, hepatoprotective, and antitumor properties (Chatterjee *et al*., 2011; Gaur & Rao, 2016; Zhao *et al*., 2020; Kui *et al*., 2021; Kumar *et al*., 2022; Roy *et al*., 2022; Ghafoor

& Niazi, 2023). Moreover, macrocybin, a natural triglyceride identified in *Macrocybe* spp., has demonstrated anticancer effects by promoting actin cytoskeleton disorganization and Caveolin-1 overexpression in tumor cells (Vilarino *et al*., 2020). In *Macrocybe crassa*, high β-glucan content, along with lipids, proteins, and phenolic compounds (e.g. benzoic acid derivatives, cinnamic acid, flavanol, pyrogallol), underlies its antioxidant, antimicrobial, anti-inflammatory, and gut microbiota-regulating properties (Acharya *et al*., 2015; Pal *et al*., 2019; Ayimbila *et al*., 2022; Cateni *et al*., 2022; Inyod *et al*., 2022). Recent studies have also underscored the therapeutic potential of *Macrocybe titans*, a species not traditionally used in folk medicine but capable of producing bioactive fucogalactans with selective cytotoxic effects. These compounds, isolated from Brazilian specimens, induce apoptosis and cell cycle arrest in breast cancer cells and inhibit melanoma cell migration without affecting normal cell viability or morphology, suggesting a targeted antitumor mechanism (Milhorini *et al*., 2018; Milhorini *et al*., 2025). In addition, *M. titans* contains β-D-glucans and α-D-glucans associated with cholesterol-lowering (Tong *et al*., 2015; Sima *et al*., 2018) and immunomodulatory effects (Felice *et al*., 2020), as well as antimicrobial activity against several human pathogens (Junior *et al*., 2021; Pereira *et al*., 2023).

Although the genus *Macrocybe* has been recognized as edible for centuries, historical documentation regarding its consumption and domestication remains scarce (Peiris *et al*., 2024). Species such as *Macrocybe gigantea*, *M. lobayensis*, and *M. crassa* are part of traditional diets in several Asian and African countries, where they are frequently sold in local markets in both fresh and dried forms (Zoberi, 1972; Rammeloo & Walleyn, 1993; Petcharat, 1996; Guissou *et al*., 2008; Dutta *and* Acharya, 2014; Kamou *et al*., 2015; Teaumroong *et al*., 2002; Pradhan *et al*., 2010). In Colombia, *M. titans* is traditionally used and recognized as an edible mushroom (Vargas *et al*., 2022), although its cultivation has not historically been practiced (Stijve, 2004). The few documented cases of artificial cultivation in *Macrocybe* species pertain primarily to *M. gigantea* (Niihara, 2002; Kinjo & Miyagi, 2006; Pamitha, 2014; Devi & Sumbali, 2021; Galappaththi *et al*., 2022; Ghafoor *et al*., 2022; Nirmala & Siva, 2023), with occasional reports also involving *M. crassa* (Inyod *et al*., 2016, 2023; Sornprasert *et al*., 2017). Nevertheless, for most *Macrocybe* species, including *M. titans*, cultivation methods remain undeveloped or anecdotal, with fruiting still primarily dependent on foraging.

Amid increasing global demand for alternative protein sources, functional foods, and sustainable agricultural practices, and thus the domestication and cultivation of native edible fungi has become increasingly relevant (Moore & Chiu, 2001; Panda *et al*., 2024). *Macrocybe titans* emerges as a promising species for cultivation due to its exceptionally large basidiomata, confirmed edibility (Li *et al*., 2021; Drewinski *et al*., 2024a) and preliminary evidence of

bioactive and nutritional potential (Carbonero *et al*., 2006; Bessette *et al*., 2007; Milhorini *et al*., 2022). Despite the availability of commercial spawn (Moore & Chiu, 2001), standardized cultivation systems and strain selection for *M. titans* are still lacking (Peiris *et al*., 2024). In Brazil, recent efforts have successfully domesticated and cultivated native wild edible mushrooms from the Atlantic Forest, demonstrating the feasibility of adapting tropical species to cultivation systems (Drewinski *et al*., 2024b, 2025). Therefore, advancing the cultivation of *M. titans* could contribute not only to the diversification of the edible mushroom sector but also to the sustainable use of native fungal biodiversity, particularly in tropical countries such as Brazil, where the native funga remains largely unexplored in the context of cultivation. In this study, we aimed to assess the cultivation potential of wild isolates of *M. titans* from Brazil and to analyze the nutritional and mineral composition of the cultivated basidiomata.

MATERIAL AND METHODS

Mushrooms collections and isolation

Six dikaryon isolates of *Macrocybe* were used in this experiment (Table 1). They were obtained from specimens collected in fragments of the Atlantic Rainforest domain, in the Brazilian states of São Paulo and Paraná. In the field, pure cultures were aseptically isolated by transferring sections of internal tissue from wild basidiomata onto potato dextrose agar (PDA) medium (Kasvi, Spain). Inoculated Petri dishes with fungal isolates were sealed with Parafilm and incubated at 25 °C in a BOD. Basidiomata were dried in a hot air dehydrator (~45 °C) and stored in hermetic plastic bags for their preservation. The vouchers are deposited at the fungarium IFungiLab (FIFUNGI) from the "Instituto Federal de Educação, Ciência e Tecnologia de São Paulo" (IFSP), and the live cultures at the "Coleção de Culturas de Algas, Fungos e Cianobactérias" (CCIBt), from the "Instituto de Pesquisas Ambientais" (IPA). This study is according to the Brazilian legislation on access to biodiversity and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen # 0000000).

Table 1. Data on the wild isolates of *Macrocybe titans* evaluated in this study.

Collector no.	CCIBt isolates[7]	Fungarium voucher	GenBank accessions		Locality in Brazil
			ITS	nrLSU	
NCC204	--	FIFUNGI 1184	**This study**	**This study**	São Paulo, SP, *CUASO*
MPD643	--	FIFUNGI 1185	**This study**	**This study**	Botucatu, SP, PP
EF67	--	Not available	**This study**	--	Cornélio Procópio, PR
EF68	--	Not available	**This study**	--	Cornélio Procópio, PR
MPCS85	--	FIFUNGI 1186	**This study**	**This study**	São Paulo, SP, DB
NCC257	--	FIFUNGI 1187	**This study**	--	São Paulo, SP, DP

PR Paraná, *SP* São Paulo, *CUASO* Cidade Universitária Armando de Salles Oliveira, *DB* Distrito de Brasilândia, *DP* Distrito de Parelheiros, *PP* Particular Property, *UA Urban Area*, *USP* Universidade de São Paulo.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from mycelial cultures grown on PDA by scraping the surface mycelium with a sterile scalpel, followed by cryogenic grinding in liquid nitrogen using a mortar and pestle. Extractions were performed with the DNeasy Plant Mini Kit (Qiagen, San Diego, California), following the manufacturer's protocol with minor modifications: the samples were incubated overnight at 65 °C after addition of Buffer AP1 and RNase A, and the DNA was eluted in 50 µl of Buffer AE. DNA concentration and purity were assessed with a NanoDrop™ Lite spectrophotometer (Thermo Fisher Scientific, Madison, Wisconsin).

The internal transcribed spacer region (ITS) was amplified using ITS1-F/ITS4 or ITS1/ITS4-B primers (White *et al*., 1990; Gardes & Bruns, 1993), and partial sequences of the nuclear ribosomal large subunit (nrLSU) were obtained using LR0R/LR5 or LR0R/LR7 (Vilgalys & Hester, 1990; James *et al*., 2006). PCR amplifications were conducted using a Mastercycler Nexus GX2 thermal cycler (Eppendorf SE, Hamburg, Germany), following the protocol described by Coelho-Nascimento *et al*. (2024).

PCR products were visualized on 2% agarose gels stained with SafeDye Nucleic Acid Stain (Cellco Biotech, São Paulo, Brazil) and purified using the QIAquick PCR Purification Kit (Qiagen) or the DPK-106 PCR Purification Kit (Cellco Biotech), following the respective manufacturer's instructions.

Taxon sampling, alignment, and phylogenetic analyses

Raw bidirectional chromatograms were assembled and curated using Geneious Prime v2024.0.5 (Biomatters Ltd., Auckland, New Zealand). Terminal nucleotide positions with low

[7] O depósito dos isolados vivos na CCIBt (IPA), bem como a atribuição dos respectivos números de acesso, ocorrerá por ocasião da submissão do manuscrito final para publicação.

Phred quality scores were manually trimmed to ensure sequence accuracy. Sampling for the phylogenetic backbone of *Macrocybe* followed established taxonomic frameworks proposed by Razaq *et al*. (2016), Vizzini *et al*. (2020), and Drewinski *et al*. (2024a).

In this study, six ITS and three nrLSU sequences were obtained. However, only ITS sequences were included in the phylogenetic analyses. Additional sequences were retrieved from GenBank to complement the dataset, comprising representatives of 43 specimens assigned to the Callistosporiaceae. A sequence of *Tricholoma palustre* A.H. Sm. was selected as the outgroup based on its phylogenetic distance from the ingroup taxa and its prior use in related studies (Vizzini *et al*. 2020). All new sequences from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/). Table 2 gives all taxa, collection information, and GenBank numbers of sequences used in this study. The selected sequences were aligned using MAFFT v.7490 (Katoh *et al*., 2002; Katoh & Standley, 2013), as implemented in Geneious Prime, applying the E-INS-i algorithm. Ambiguously aligned regions were manually curated and excluded from downstream analyses, and indels were treated as missing data.

To verify the identity of the newly generated sequences and to assess species boundaries within *Macrocybe*, a dataset comprising ITS sequences was analyzed independently. Phylogenetic inference was conducted using both Maximum Likelihood (ML) and Bayesian Inference (BI) frameworks via the CIPRES Science Gateway platform (https://www.phylo.org/portal2/home.action) (Miller *et al*., 2010). ML analyses were performed with RAxML-HPC v.8 on XSEDE (v.8.2.12) (Stamatakis, 2014) under the GTRCAT approximation model, implementing 25 rate categories per site and 1,000 rapid bootstrap replicates (Felsenstein, 1985) to assess branch support.

Bayesian analyses were conducted with MrBayes v.3.2.7a on XSEDE (Huelsenbeck & Ronquist, 2001; Ronquist *et al*., 2012), with the optimal nucleotide substitution model selected using jModelTest2 v.2.1.6 (Posada & Crandall, 1998) based on the Bayesian Information Criterion (BIC). Four independent Markov chain Monte Carlo (MCMC) runs were executed for 10 million generations, sampling every 1,000th generation to yield a total of 10,000 trees. The initial 25% of trees were discarded as burn-in, and the remaining trees were used to compute a majority-rule consensus tree and Bayesian posterior probability (BPP) values. Convergence diagnostics were assessed by evaluating the average standard deviation of split frequencies (< 0.01) and effective sample sizes (ESS) were inspected in Tracer v.1.6 (Rambaut *et al*., 2018) to confirm run stationarity and parameter convergence.

Table 2. Taxa, vouchers, locations, and GenBank accession numbers of the DNA sequences used for the phylogenetic analyses treated in this study. Sequences in bold were newly generated.

Species/name	Sample no.	Locality	GenBank accessions
			ITS
Anupama indica	10031 (AMH)	India	MH989588
A. indica	10033 (AMH)	India	MH989590
Callistosporium elegans	013382 (CORT)	Puerto Rico	MN017508
C. elegans	013860 (CORT)	Dominican Republic	MN017509
Guyanagarika aurantia	070902 (TENN-F)	Guyana	KX092078
G. aurantia	070903 (TENN-F)	Guyana	KX092079
Macrocybe crassa	024256 (SFSU-F)	Thailand	MN017540
M. crassa	DOA 01	Thailand	LC029416
M. crassa	141390 (K-M)	Thailand	MN017541
M. crassa ***	10295 (AMB)	Seychelles	MN017539
Macrocybe gigantea	ALV14683	Australia	MH014646
M. gigantea	Kerala-Mg	India	KY744346
M. gigantea	NEHU ARD 5	India	KM983610
M. gigantea	MGLH	China	KJ463730
M. gigantea	MGNBH	China	KJ463731
*M. gigantea****	SCAU 1	China	JX041888
M. gigantea ***	SCAU 2	China	JX068526
M. gigantea	--	India	JN192443
M. gigantea	--	India	JN006792
M. gigantea	TrG1	Iran	MG867660
Macrocybe sp.	iNAT:31963466	USA	MW018915
Macrocybe sp.	DOA 04	Thailand	LC029418
Pseudolaccaria pachyphylla	gmb00672 (TR)	Italy	KR818912
P. pachyphylla	0066637 (GB)	Sweden	KU058504
Macrocybe sardoa	29083a (MCVE) **holotype**	Italy	MN017542
M. sardoa	29083b (MCVE) **holotype**	Italy	MN017543
*M. sardoa****	MCVE n degrees 29083	Italy	MF401956
M. sardoa	KUBOT-KRMK-2020-01	India	MT880333
*M. sardoa****	SCRIPCOG69	India	PV037429
*M. sardoa****	MDU 2022	India	OQ998336
Macrocybe titans	MPCS85	Brazil	OR166790
M. titans	MDX20221	Brazil	OR166791
M. titans	NCC204	Brazil	**Table 1**
M. titans	MPD643	Brazil	**Table 1**
M. titans	EF67	Brazil	**Table 1**
M. titans	EF68	Brazil	**Table 1**
M. titans	NCC257	Brazil	**Table 1**
M. titans	mt3	Brazil	MZ519068
M. titans	55023 (K-M)	Puerto Rico	MN017544
M. titans	58974 (FLAS-F)	USA	MN017545
M. titans	59217 (FLAS-F)	USA	MN017546
M. titans	61267 (FLAS-F)	USA	MH211846
M. titans	127429 (JBSD)	Dominican Republic	MN017547
M. titans	127842 (K-M)	USA	MN017548

M. titans	iNat # 191315990	USA	PV223613
M. titans	--	Italy	LR992036
Xerophorus olivascens	18226 (AMB)	Italy	MN017558
X. dominicanus	127428 (JBSD) **holotype**	Dominican Republic	MN017550
Outgroup			
Tricholoma palustre	PBM 2494 (CUW)	USA	DQ494699
Tricholoma sp.	iNaturalist # 132756632	USA	OP643484

Note: *** indicates taxon names modified according to Vizzini *et al*. (2020).

Mycelial growth at different temperatures

Six wild isolates of *Macrocybe titans* were assessed for their mycelial performance on PDA medium under a range of incubation temperatures. Sterile PDA (30 mL) was poured into 90 mm diameter Petri dishes under laminar flow conditions. A 9.6 mm plug of actively growing mycelium, excised from the margin of a previous pure culture, was centrally inoculated onto the surface of the culture media of each plate. The plates were incubated in a BOD at four different temperatures (20 °C, 25 °C, 30 °C, and 35 °C) (Thongbai *et al*., 2017). Growth was monitored daily, and the final colony diameter was recorded at the point when at least one isolate reached the edge of the plate. For dry biomass determination, colonized PDA was liquefied by microwave treatment (30 seconds at full power) to release the mycelium (Vargas-Isla & Ishikawa, 2008). The fungal biomass was then recovered by vacuum filtration using qualitative filter paper, rinsed thoroughly with sterile distilled water to remove residual medium, and subsequently dried in an oven at 50 °C until constant weight was achieved (Vargas-Isla & Ishikawa, 2008). The weight was recorded on an electronic scale (gram). Each treatment was conducted with ten biological replicates.

Evaluation of two cereal grains for spawn production

The isolate exhibiting the highest radial mycelial growth and dry biomass yield under the temperature optimization assays was selected for subsequent spawn production. Two cereal grain substrates were tested to assess their suitability for supporting mycelial colonization: wheat (*Triticum aestivum* L.) and red sorghum [*Sorghum bicolor* (L.) Moench]. These grains were selected due to their wide availability, low cost, and ease of acquisition in the local market, particularly in the “Zona Cerealista” of São Paulo city, a central wholesale hub for cereals and agricultural inputs in Southeastern Brazil. Spawn production followed the protocol of Thongbai *et al*. (2017), with minor modifications. Grains were manually cleaned to remove impurities and broken kernels, soaked in distilled water overnight (12–14 hours), rinsed, and boiled for 15

–20 minutes until softened but intact. After cooling to room temperature, excess water was drained to reach field capacity, and 2% calcium sulfate (gypsum) was added to improve aeration and prevent clumping. Approximately 400 g of grain spawn substrate were dispensed into 600 mL glass jars, closed with cotton plugs and aluminum foil, and autoclaved twice at 121 °C for 90 min, with cooling to room temperature between cycles. After sterilization, jars were shaken while warm to loosen the substrate and then cooled aseptically. Each jar was inoculated with three 8 mm agar plugs taken from the actively growing margin of PDA pure cultures. Inoculated jars were incubated at 25 °C in the dark in a BOD chamber. Mycelial growth was monitored daily by measuring the vertical advancement of the colonization front from three fixed reference points using a digital caliper. Each treatment included ten biological replicates.

Compost substrate preparation, casing layer, and primordia induction

Compost substrate preparation was conducted by "CG Compostagem", a specialized company in phase-based substrate production for mushroom cultivation, located in Mogi das Cruzes, São Paulo, Brazil. The lignocellulosic base substrate consisted of 60 kg of sugarcane bagasse (*Saccharum officinarum* L.) and 40 kg of brachiaria straw (*Urochloa* spp.), totaling 100 kg of dry matter (DM) (Figure 1). The substrate was subsequently supplemented with chicken manure (40 kg), cottonseed meal (5 kg), calcium carbonate (3 kg), gypsum (5 kg), and ammonium sulfate (1.5 kg).

Compost preparation followed a semi-controlled protocol adapted from commercial *Agaricus* cultivation practices (Pardo-Giménez *et al*., 2014; Pardo *et al*., 2017; Thai *et al*., 2022) and comprised three sequential stages organized into two main phases (Figure 1):

1. Phase I (initial aerobic composting, 10 days): Raw materials were thoroughly mixed and composted under aerobic conditions to initiate the degradation of lignocellulosic substrates.
2. Phase I (active thermophilic composting, 12 days): The compost was maintained in open piles with mechanical turning every two days to ensure aeration and homogeneous microbial decomposition.
3. Phase II (pasteurization and conditioning): The compost was transferred to a pasteurization tunnel and subjected to peak heating at 57–60 °C for 6–10 h for pasteurization. Subsequently, the temperature was reduced and maintained at 45–48 °C for 3–5 days, which could be extended up to ~7 days, for conditioning, allowing

ammonia dissipation and the establishment of a thermophilic microbiota conducive to subsequent fungal colonization.

Throughout the composting process, moisture content was kept at optimal levels (65–75%), and internal temperatures were recorded daily using digital probes inserted into the center of the piles. After pasteurization, the compost was allowed to cool gradually to ambient temperature before inoculation.

Figure 1. Compost substrate preparation for mushroom cultivation. **A**. Brachiaria straw. **B**. Sugarcane bagasse. **C**. Compost undergoing primary fermentation. **D**. Pasteurization tunnel. **E**. Pasteurized compost packed in bags. Photos by Coelho-Nascimento CC.

Four kilograms of compost substrate were loaded into 15 L polypropylene cultivation bags (40 × 35 × 20 cm), filled to ca. half of their nominal volume. Inoculation was performed using 2% spawn prepared from red sorghum. Spawn was applied by alternating layers of compost and throughout the bag to ensure uniform mycelial distribution. The bags were incubated at 30 °C under 60–70% relative humidity in darkness to promote colonization. Bag surfaces were perforated at the onset of incubation using a sterile manual punch to produce holes of approximately 0.30 mm in diameter, thereby enhancing gas exchange. The substrate medium was allowed to become fully colonized before casing. A mixture of 30% peat moss (*Sphagnum* spp.), 40% iron-rich red sandy soil, 10% chopped rice straw, and 20% vermiculite was pasteurized at 60 °C for four hours and used as a casing material. After full colonization of

the compost, the upper portion of each bag was opened, and a 1.5-inch layer of casing was evenly applied over the surface. Once the mycelium had reached the surface of the casing layer, environmental conditions were modified to induce primordia formation. The cultivation chamber was maintained at a mean temperature of 24 °C (range: 22–26 °C), with CO_2 concentrations near 650 ppm (range: 570–800 ppm), and humidity close to 90% (range: 85–95%). Humidity was regulated via an automated misting system and supplemented with manual spraying when necessary. The experiment was carried out with 12 replicates.

Harvest and yield parameters of cultivated mushrooms

Mushroom development was monitored daily throughout the cropping period. Quantitative and qualitative parameters were recorded as follows: (1) cumulative fresh weight of mushrooms harvested from each cultivation bag across successive flushes; (2) morphological quality traits, including pileus diameter, stipe diameter, and stipe length; (3) total mushroom yield (MY); and (4) biological efficiency (BE).

Cultivation bags were evaluated for a period of 60 days following primordia induction. Mature mushrooms were harvested, weighed, and subsequently dried in a hot air dehydrator (~45 °C). The time to primordia formation and the onset of the first harvest were documented.

Mushroom yield (MY) was expressed as the proportion of total fresh biomass obtained in relation to the fresh weight of the substrate, using the following formula (Peksen & Yakupoglu, 2008):

$$MY\ (\%) = \frac{\text{Total fresh mushroom weight (g)}}{\text{Fresh substrate weight (g)}} \times 100$$

Biological efficiency (BE), indicating the conversion efficiency of substrate into fungal biomass, was calculated according to (Fan *et al*., 2000):

$$BE\ (\%) = \frac{\text{Total fresh mushroom weight (g)}}{\text{Dry substrate weight (g)}} \times 100$$

Proximate nutritional analysis

Proximate analysis of the basidiomata produced by isolate MPD643 was conducted at the Bromatology Laboratory of the Animal Production Department at ‘Universidade Estadual

Paulista Julio de Mesquita Filho' – UNESP, in Dracena (Sao Paulo state, Brazil) according to the methodologies of the Adolfo Lutz Institute (https://www.ial.sp.gov.br/). The moisture content was determined by dehydrating the samples in an oven with forced air circulation at 105 °C for 12 h or until constant weight (method 012/IV) (Zenebon *et al*., 2008). Crude fibre was determined by acid and alkaline digestion and subsequent heating of 2 g sample in a Thermolyne® Small Benchtop Muffle (Thermo Fisher Scientific Inc., Waltham, USA) at 550 ºC to produce ash of gray color. The desiccator was used for cooling, and the sample was weighed (method 044/IV) (Zenebon *et al*., 2008). The ash content was determined by subjecting a 3 g sample to incineration in a muffle furnace (branded above) at 550 °C for six h (method 018/IV) (Zenebon *et al*., 2008). Crude protein was evaluated by the macro-Kjeldahl apparatus (method 036/IV, Zenebon *et al*., 2008) using the conversion factor of 4.38 [Crude protein (%) = 4.38 × Nitrogen (%)], which is used for fungi (Crisan & Sands, 1978; Krishnamoorthi *et al*., 2022). Soxhlet extraction with petroleum ether was used for the gravimetric evaluation of fat content (method 321/IV, modified). The total of carbohydrates was calculated by the difference method as follows: carbohydrates = 100 - (moisture + ash + crude fat + crude protein + crude fiber) (Colak *et al*., 2007). Determination of total energy value was according to the given equation: energy (kcal) = 4 × (g crude protein + g carbohydrate) + 9 × (g crude fat) (Grimm *et al*., 2021; Lee & Cho, 2021). All experiments were carried out in triplicate. The results were expressed in dry weight percentage and presented as the mean ± standard deviation of the three parallel measurements.

Determination of mineral elements

The estimation of the mineral content of samples previously subjected to proximate analysis was conducted at the Laboratory of Plant Nutrition at the Faculty of Engineering at 'Universidade Estadual Paulista Júlio de Mesquita Filho' – UNESP, in Ilha Solteira city (São Paulo state, Brazil). The content of macrominerals (P, K, Ca, and Mg) and microelements (Cu, Fe, Mn, and Zn) was determined using spectrophotometric methods according to Malavolta *et al*. (1997) and Silva (2009).

Statistical analyses

All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software Inc., San Diego, USA). Normality of the data was assessed with the Shapiro–Wilk test, and an insignificant result at a 95% confidence level indicated that the assumptions of

normality required for parametric analyses were met (Shapiro & Wilk, 1965). Based on this result, a two-way analysis of variance (two-way ANOVA) was used to evaluate the effects of the experimental treatments and their interaction on the measured variables. When significant differences were detected ($p < 0.05$), Tukey's multiple comparison test (Tukey, 1959) was applied to discriminate between treatments.

RESULTS

Molecular phylogenetic analyses

No topological inconsistency was detected between the ML and BI analyses. The phylogenetic tree inferred from the ML strategy is presented, with statistical results from both ML (Bootstrap Supports, BS) and BI (Bayesian Posterior Probabilities, BPP) displayed on the branches (Figure 2). The best-fit model for BI analyses was the GTR + I + G model. After excluding the ambiguous positions, the data matrix of the ITS region consisted of 715 bp, of which 338 were conserved, 316 were parsimony-informative, and 61 were variable but parsimony-uninformative.

The inferred phylogeny was largely congruent with the arrangement outlined by Vizzini *et al*. (2020), which recognized *Callistosporiaceae* as a distinct, monophyletic lineage within the Tricholomatineae. All newly generated ITS sequences from wild isolates of *Macrocybe titans* consistently clustered with reference sequences of this species from the Dominican Republic and the USA, including Puerto Rico (BP = 100%; BPP = 1.00), thereby confirming their taxonomic placement and supporting the interpretation of the American populations of *M. titans* as a well-delimited lineage. This clade was recovered as sister to a clade comprising *M. sardoa* and to an internally homogeneous and genetically distinct lineage containing sequences assigned to *M. crassa* and *M. gigantea*, with strong statistical support (BP = 99%; BPP = 1.00). These findings converge to support the treatment of *M. gigantea* as a later synonym of *M. crassa*, which holds nomenclatural priority (**Vizzini *et al*., 2020**).

Mycelial growth at different temperatures

Temperature emerged as a pivotal environmental parameter regulating mycelium growth of fungi. All isolates exhibited normally distributed radial growth and biomass accumulation data, allowing for statistical comparison of growth temperatures using Tukey's test.

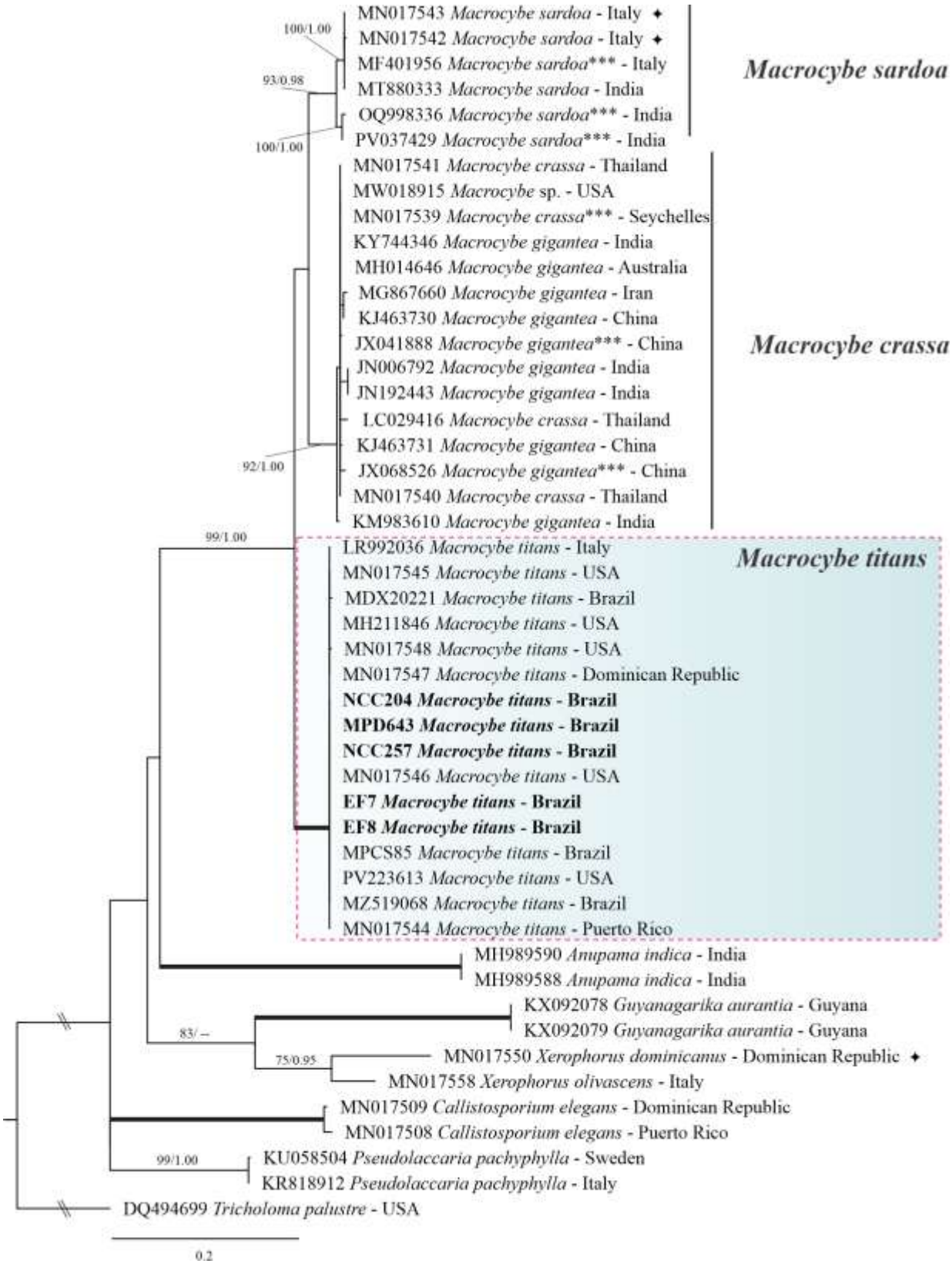

Figure 2. ITS-based phylogram from ML analysis of *Callistosporiaceae*. Branches are annotated when supported by ≥70% bootstrap (BS; left) or ≥0.95 Bayesian posterior probability (BPP; right). Thick branches indicate maximum support (BS = 100; BPP = 1.00). The *Macrocybe titans* clade is highlighted in color. Collections sequenced in the present study are shown in boldface. Black stars indicate holotype specimens. Asterisks (***) denote taxon names modified according to Vizzini *et al*. (2020).

All isolates grew vigorously at both 25 °C and 30 °C, with no significant differences in mycelial diameter between these temperatures (Figure 3 and 6A–D). The isolate MPD643 was the first to achieve complete plate colonization, reaching the perimeter within 7 days at both 25 °C and 30 °C. In contrast, dry biomass accumulation varied significantly between 25 °C and 30 °C (Figure 4), with the isolate MPD643 yielding the highest dry mycelial biomass (304 mg ± 21.3) at 30 °C, followed closely by the isolate EF67 (278 mg ± 12.9), although this difference was not statistically significant ($p > 0.05$).

Based on the results of the temperature trials, the wild isolate MPD643 was selected for subsequent experiments on spawn production and substrate colonization at 30 °C.

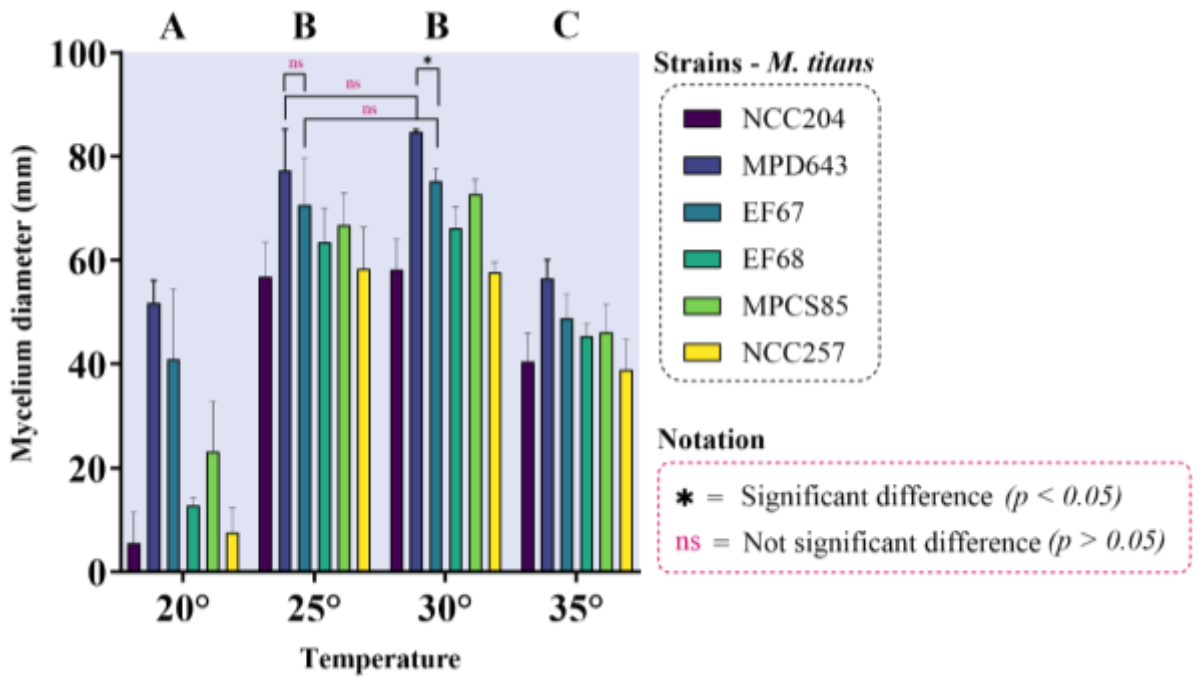

Figure 3. Effects of temperature on the mycelial diameter (mm) of six wild isolates of *Macrocybe titans* on the seventh day of incubation on PDA medium. Capital letters indicate comparisons among the means of all isolates across different temperatures. Asterisk denotes statistically significant differences (Tukey's test, $p < 0.05$) highlighting the best-performing values of the isolates at the best temperatures.

Evaluation of two cereal grains for spawn production

The colonization dynamics of the active mycelium (isolate MPD643 previously cultured on PDA medium) were evaluated on cereal grains (sorghum and wheat) incubated at 30 °C. Sorghum supported the most rapid mycelial proliferation, with visible colonization initiating on the third day and reaching a mean daily linear extension of 15.65 mm ± 2.16. Complete sorghum colonization was achieved within nine days. In contrast, wheat grains exhibited a delayed onset of colonization, beginning on the fourth day requiring 14 days for full substrate

colonization, with a lower average daily growth rate of 9.47 mm ± 1.56. Statistical analysis confirmed that sorghum significantly outperformed ($p < 0.05$) wheat as a spawning substrate for promoting vigorous mycelial development of *Macrocybe titans* (Figure 5). Consequently, sorghum was selected for subsequent spawn production in the cultivation experiment.

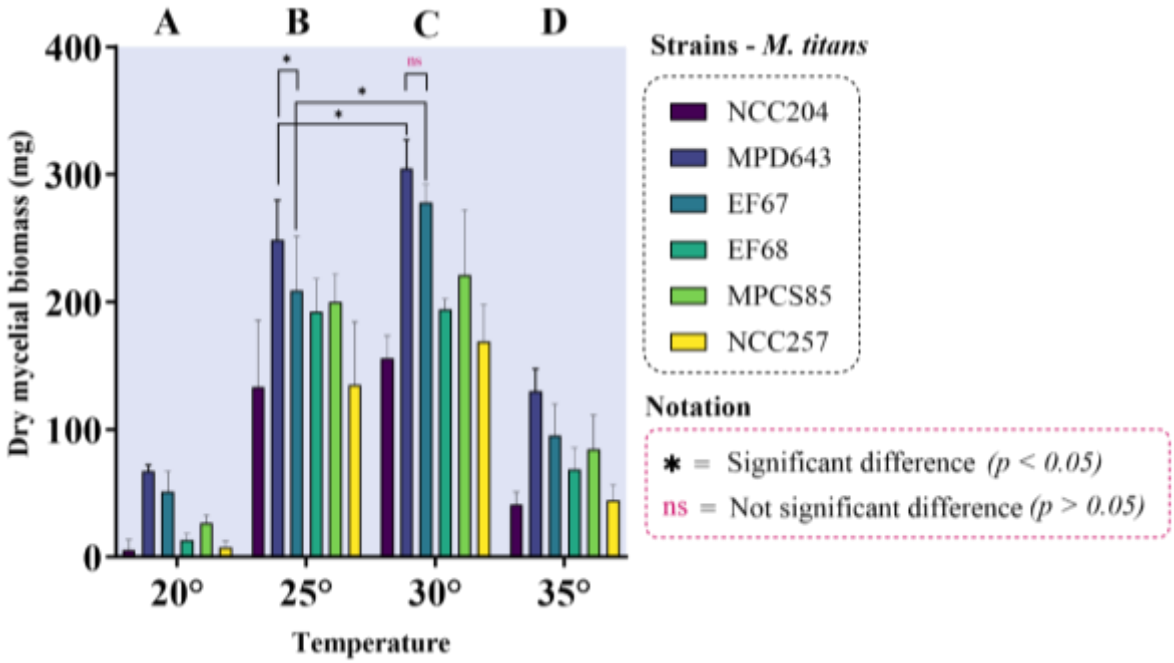

Figure 4. Effects of temperature on the dry mycelial biomass (mg) of six wild isolates of *Macrocybe titans* on the seventh day of incubation on PDA medium. Capital letters indicate comparisons among the means of all isolates across different temperatures. Asterisks denote statistically significant differences (Tukey's test, $p < 0.05$) highlighting the best-performing values of the isolates at each better temperature.

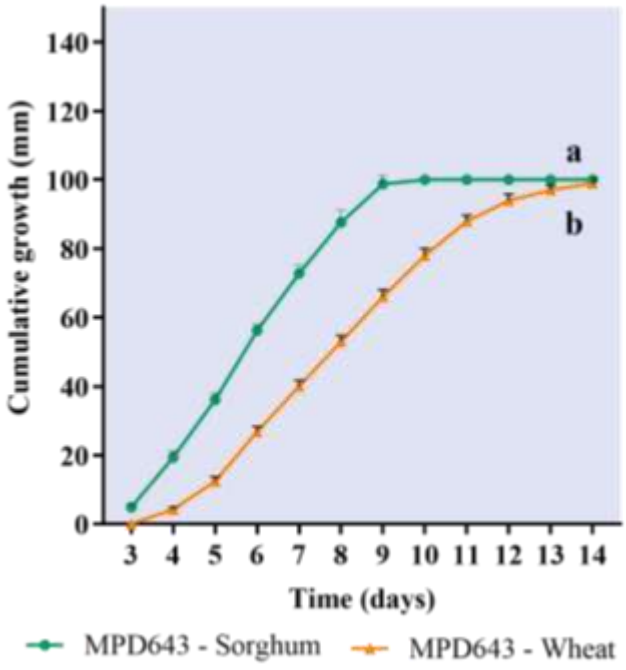

Figure 5. Cumulative daily linear extension of *Macrocybe titans* isolate MPD643 on sorghum and wheat grains. Different letters represent significant differences (Tukey–Kramer test after ANOVA, $p < 0.05$).

Harvest and yield parameters of cultivated mushrooms

Spawn run began uniformly across all substrate bags on the fourth day of incubation. Complete colonization of the compost substrate by the wild isolate MPD643 was achieved within 32–38 days at 30 °C (Figure 6). Primordia formation was observed on the casing surface after 9–25 days under a mean temperature of 24 °C (Figure 6). The first mature basidiomata were harvested 21–28 days after casing. A subsequent flush followed after 32–41 days, and the final flush was completed at 45–51 days. The average mushroom yield of the first two flushes was 18.64% ± 0.03 , and the biological efficiency was 62.23% ± 0.10. The third flush was excluded from productivity assessments due to the high incidence of primordia abortion and morphological abnormalities in mature basidiomata, such as stipe elongation and malformation, which were attributed to technical issues in the cultivation chamber.

Detailed observations were made on yield-related morphological parameters of mature basidiomata, including total fresh weight, pileus diameter, stipe length, and stipe width. Individual fresh weights ranged from 590 to 915 g (mean ± SD: 746 ± 115.35 g). The pileus diameter varied between 10 and 18 cm (mean ± SD: 14.2 ± 2.84 cm). Stipe length ranged from 8 to 13 cm (mean ± SD: 10.4 ± 1.79 cm), and stipe diameter varied from 2.5 to 6 cm (mean ± SD: 4 ± 1.17 cm). The basidiomata successfully cultivated from the MPD643 wild isolate are shown in Figure 6, whereas Figure 7 displays the original specimen as found in their natural habitat. The nutritional and mineral compositions of *M. titans* are shown in Table 3.

DISCUSSION

Optimal mycelial growth of the wild isolate MPD643 of *Macrocybe titans* was observed at 30 °C. To date, this is the first study to experimentally evaluate the effect of different temperatures on mycelial development in *M. titans*. Although Dương et al. (2017) reported the successful cultivation of an isolate identified as *M. titans* from Vietnam, their study focused exclusively on the testing of cultivation substrates, without performing controlled assays to assess the optimal temperature for mycelial growth. The cultivation was conducted under ambient conditions, with recorded temperatures ranging from 28 to 32 °C. However, given the geographically structured distribution of species within the genus *Macrocybe* and the recognition of *M. titans* as a Neotropical endemic (Vizzini et al. 2020), the Vietnamese isolate

likely corresponds to *M. crassa* or *M. gigantea*. Previous reports indicate that *M. crassa* has been successfully cultivated at temperatures ranging from 20 to 34 °C (Inyod *et al*., 2016; Sornprasert *et al*. 2017; Verma *et al*. 2017), and *M. gigantea* (Niihara, 2002; Devi & Sumbali, 2021a, b; Galappaththi *et al*., 2022; Ghafoor *et al*. 2022; Nirmala & Siva, 2023), the most studied species in the genus, at 25 to 35 °C. Notably, Ghafoor et al. (2022) is the only study to have experimentally determined the optimal temperature for mycelial growth in *M. gigantea*, reporting the highest performance on PDA at 30 °C. Altogether, the evidence supports that *Macrocybe* isolates grow well at temperatures of 25 °C or higher, reinforcing the feasibility of cultivating this genus under tropical and subtropical conditions.

Figure 6. Mycelial development and cultivation stages of the wild isolate MPD643 of *Macrocybe titans*. **A**–**D**. Radial growth on PDA medium after seven days of incubation at different temperatures: **A**. 20 °C. **B**. 35 °C. **C**. 25 °C. **D**. 30 °C. **E**. Compost substrate fully colonized prior to casing. **F**. Emergence of primordia. **G**. Young mushrooms. **H**. Mature mushrooms ready for harvest. **I**. Harvest mushrooms. Photos by Coelho-Nascimento CC.

Mycelial growth of the wild isolate was assessed on sorghum and wheat grains. Sorghum supported faster and more vigorous colonization (mean daily linear extension: 15.65 ± 2.16 mm/day) and was also more cost-effective than wheat (Notícias Agrícolas, 2025), in addition to being widely available in southeastern Brazil. Ghafoor et al. (2022) also identified sorghum at 30 °C as the optimal substrate for spawn production of a wild *Macrocybe gigantea* isolate from Pakistan. These findings support the use of sorghum as a reliable substrate for spawn preparation, consistent with its widespread application in the commercial cultivation of other edible fungi, including species of *Macrocybe* (Ogden & Prowse 2004).

This study showed that *M. titans* collected in Atlantic rainforest could be domesticated and brought into cultivation successfully. The wild isolate MPD643 of *Macrocybe titans* achieved a BE of 62.23 ± 0.10 % when cultivated on a compost-based substrate composed primarily of sugarcane bagasse and *Brachiaria* straw. Both materials are lignocellulosic agricultural wastes that are readily available and low-cost (Rodrigues *et al*., 2021; Ajala *et al*., 2021), especially in Southeastern Brazil that is characterized by intensive agricultural and livestock activity (Alves *et al*., 2023). Reports on BE from the cultivation of wild *Macrocybe* isolates are currently restricted to *M. crassa* and *M. gigantea* (Peiris *et al*., 2024). For *M. crassa*, BE ranged from 34.16% to 65.89%, with the highest values obtained on composts formulated with spent *Pleurotus eryngii* substrate at 25–30 °C (Inyod *et al*., 2016; Inyod *et al*., 2023). *M. gigantea*, in turn, has shown BE values between 17.3% and 86.77%, with the best performance observed on mixed substrates of wheat straw and tea waste at 30 °C (Niihara, 2002; Devi & Sumbali, 2021a, b; Galappaththi *et al*., 2022; Ghafoor *et al*. 2022; Nirmala & Siva, 2023). It is noteworthy that *M. gigantea* cultivated on agricultural wastes (viz. bajra stalk, maize stalk/cobs, paddy straw, and wheat straw) by Devi and Sumbali (2021a, b) yielded biological efficiency (BE) values of 64–68.7% and 60.6–70.2%, respectively, which closely align with those obtained for *M. titans* in the present study.

Regarding the proximate composition of the wild isolate *Macrocybe titans* MPD643, carbohydrates represented the major component (52.9% ± 0.04), followed by crude protein (18.33% ± 0.20), crude fibre (14.23% ± 0.18), crude fat (7.57% ± 0.01), and ash (6.97% ± 0.01). The high carbohydrate content suggests that this species is a valuable source of dietary energy, consistent with values reported for other *Macrocybe* species, which range from 32% to 75.75% (Inyod *et al*., 2016; Das, 2017; Ayimbila *et al*., 2022; Khumlianlal *et al*., 2022; Inyod *et al*., 2023; Chibou Ousmane *et al*., 2024). The crude protein content observed in *M. titans* is comparable to that reported for *M. crassa* (14.3%; Ayimbila *et al*., 2022), *M. gigantea* (16.7%; Giri *et al*., 2013), and *M. lobayensis* (20.66%; Chibou Ousmane *et al*., 2024). However, substantially higher protein levels have also been recorded for *M. crassa* and *M. gigantea*, with

values ranging from 30.89% to 37.60% (Liu *et al*., 2007; Das, 2017; Khumlianlal *et al*., 2022; Inyod *et al*., 2023).

Figure 7. Basidiomata of *Macrocybe titans* from collection MPD643, found in Botucatu, São Paulo, Brazil. Photos by Vitali A.

The crude fibre content determined for *M. titans* (14.23% ± 0.18) is notably higher than the values reported for other species of the genus, which range from 1.67% to 12.5% (Liu *et al.*, 2007; Giri *et al.*, 2013; Inyod *et al.*, 2016; Das, 2017; Ayimbila *et al.*, 2022; Khumlianlal *et al.*, 2022; Inyod *et al.*, 2023). Such elevated fibre content may contribute to the functional and nutritional value of this species. As for crude fat, edible mushrooms typically present contents ranging from 1% to 15% on a dry weight basis (Sande *et al.*, 2019). The relatively low fat content observed in *M. titans* (7.57% ± 0.01) aligns with values reported for other *Macrocybe* species, which range from 1.27% to 4.89% (Liu *et al.*, 2007; Giri *et al.*, 2013; Inyod

et al., 2016; Das, 2017; Ayimbila *et al.*, 2022; Galappaththi *et al.*, 2022; Khumlianlal *et al.*, 2022; Inyod *et al.*, 2023; Chibou Ousmane *et al.*, 2024).

Table 3. Proximate and mineral composition of the wild isolate MPD643. Values are expressed in dry weight basis and presented as mean ± standard deviation of three replicates.

***M. titans* wild isolate MPD643**		
Energy	(Kcal/100 g)	353.03 ± 0.74
Proximate composition		
	Ash (%)	6.97 ± 0.01
	Crude protein (%)	18.33 ± 0.20
	Carbohydrates (%)	52.9 ± 0.04
	Crude fibre (%)	14.23 ± 0.18
	Crude fat (%)	7.57 ± 0.01
Macrominerals		
	K (mg/kg)	31,033 ± 817.9
	Mg (mg/kg)	1613 ± 49
	P (mg/kg)	11,250 ± 736
	Ca (mg/kg)	213 ± 12.5
Microminerals		
	Fe (mg/kg)	102 ± 4.32
	Mn (mg/kg)	5.7 ± 1.25
	Cu (mg/kg)	94.7 ± 12.26
	Zn (mg/kg)	60.3 ± 8.99

The mineral profile of basidiomata from the wild isolate MPD643 showed potassium (K) as the most abundant macroelement (31,033 mg/kg ± 817.9), followed by phosphorus (P; 11,250 mg/kg ± 736), magnesium (Mg; 1,613 mg/kg ± 49), and calcium (Ca; 213 mg/kg ± 12.5). The elevated potassium content is consistent with typical mineral patterns in edible mushrooms, where this element predominates and plays a key role in osmotic balance and cellular function (Chang & Miles, 2004). Among the microminerals, iron (Fe) was present at the highest level (102 mg/kg ± 4.32), followed by copper (Cu; 94.7 mg/kg ± 12.26), zinc (Zn; 60.3 mg/kg ± 8.99), and manganese (Mn; 5.7 mg/kg ± 1.25). These micronutrients are essential cofactors in metabolic and enzymatic processes, and the concentrations observed in *M. titans* are comparable to those reported for cultivated edible fungi (Vetter, 2019)

This study demonstrated the successful cultivation of a wild isolate of *M. titans* collected from the Atlantic Rainforest of Brazil, marking an initial step toward its potential domestication. The species adapted well to controlled conditions and showed promising biological efficiency on affordable, regionally available lignocellulosic substrates. While these results are encouraging, further efforts are needed to deepen our understanding of its cultivation biology, optimize production conditions, and evaluate the nutritional and technological qualities of the fruiting bodies. As a native and underexplored species, *M. titans* may offer new

opportunities for diversified mushroom production in tropical regions, especially when aligned with low-cost, sustainable practices.

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Considerações finais

Esta tese buscou contribuir para o conhecimento sobre cogumelos comestíveis da Mata Atlântica A pesquisa concentrou-se nos gêneros *Macrolepiota*, *Pseudohydnum*, *Tuber* e *Macrocybe*, revelando uma diversidade ainda pouco conhecida de macrofungos com potencial alimentício. Ao longo do trabalho, foram descritas novas espécies e variedades, identificados usos tradicionais, realizados testes de cultivo, e analisada a composição nutricional de cogumelos comestíveis silvestres. Esses resultados fornecem fundamentos consistentes para o uso e valorização de espécies nativas.

Para o gênero *Macrolepiota*, foram descritas quatro novas espécies (*M. capelariae, M. abruptibulbosa, M. chapeletae*, e *M. pernudae*) e duas novas variedades (*M. capelariae* var. *velana* e *M. pulchella* var. *gymnopodia*). Três espécies já conhecidas para o Brasil foram revistas e confirmadas por filogenia molecular: *M. bonaerensis*, *M. sabulosa* e *M. cyanolamellata*. As análises nutricionais de *M. bonaerensis*, *M. capelariae* e *M. chapeletae* evidenciaram perfis consistentes e promissores. Os teores de proteína bruta variaram de 30.65% em *M. bonaerensis* a 35,48% em *M. chapeletae*, enquanto os de fibra alimentar oscilaram entre 14.34% e 15.86%. O potássio destacou-se como o mineral mais abundante, com valores entre 1.931,67 e 2.003,33 mg/100 g, seguido por fósforo (983.33 a 1.383,33 mg/100 g) e magnésio (92 a 132 mg/100 g). Esses valores indicam o potencial dessas espécies como fontes alimentares relevantes, comparáveis aos cogumelos cultivados mais consumidos.

Para o gênero *Pseudohydnum*, foram descritas quatro espécies novas: *P. brasiliense*, *P. brunneovelutinum*, *P. cupulisnymphae* e *P. viridimontanum*. Trata-se dos primeiros registros completos do gênero para a região neotropical. A comestibilidade de *P. brasiliense* também foi documentada, indicando seu uso potencial como alimento.

Em relação ao gênero *Tuber*, foi descrita uma nova espécie, *T. bianchettiformis*, e registradas pela primeira vez no Brasil *T. lyonii*, *T. brennemanii*, e *T. maculatum*. As duas primeiras também representam os primeiros registros para o Hemisfério Sul. A presença de espécies trufas verdadeiras adaptadas às condições climáticas brasileiras abre novas perspectivas para o cultivo de trufas em escala local.

O cultivo bem sucedido de um isolado MPD643 de *Macrocybe titans*, obtido a partir de um espécime coletado na Mata Atlântica, demonstrou o potencial agronômico da espécie. O isolado apresentou melhor crescimento micelial em meio BDA a 30ºC e melhor desempenho em grãos de sorgo na produção de inóculo. O cultivo ocorreu em substrato compostado a base de bagaço de cana e palha de braquiária, atingindo eficiência biológica de 62,23%. Os basidiomas cultivados apresentaram teor médio de proteína bruta de 15,3%, valor compatível

com o de cogumelos comestíveis amplamente cultivados. O potássio foi o mineral predominante, com concentração de 31.033 mg/kg, seguido por fósforo (11.250 mg/kg), magnésio (1.613 mg/kg) e cálcio (213 mg/kg). Entre os microminerais, os maiores teores foram observados para ferro (102 mg/kg), cobre (94,7 mg/kg) e zinco (60,3 mg/kg), enquanto o manganês foi detectado em menor quantidade (5,7 mg/kg). Esse perfil nutricional evidencia que *M. titans* não apenas possui viabilidade de cultivo em condições controladas, como também apresenta alto valor nutritivo, sendo uma opção viável para diversificação alimentar e inserção em cadeias produtivas voltadas à produção de cogumelos com qualidade funcional.

Além das contribuições taxonômicas e experimentais, esta tese destaca a relevância dos conhecimentos tradicionais associados ao uso de cogumelos silvestres no Brasil. As informações obtidas revelam práticas alimentares e sistemas de classificação popular ainda pouco documentados, especialmente em comunidades rurais do Sul e Sudeste. O reconhecimento desses saberes fortalece ações de conservação e valorização da biodiversidade local.

Em síntese, esta tese integra diferentes áreas do conhecimento e contribui para o reconhecimento da funga comestível brasileira como recurso estratégico. Os dados obtidos servem de base para ações voltadas à conservação da biodiversidade, à segurança alimentar, ao desenvolvimento de sistemas produtivos regionais e à expansão da pesquisa em micologia aplicada. A partir da caracterização taxonômica, do registro de usos tradicionais, das análises nutricionais e dos testes de cultivo, este trabalho reforça a importância dos macrofungos na construção de sistemas alimentares mais sustentáveis e no uso racional da biodiversidade nacional.

ANEXO A

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Macrolepiota capelariae (Agaricaceae, Basidiomycota): a new species from the Brazilian Atlantic Rainforest with extended records to Argentina and Mexico

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Abstract

A new *Macrolepiota* species from Brazil, *M. capelariae*, is proposed here based on morphological and molecular evidence. The description and illustrations are based on specimens collected in a remnant of the Atlantic Rainforest in São Paulo, Southeast Brazil. Analyses of nrITS sequences supported the recognition of this new species, in combination with morphological evidence, and revealed its phylogenetic placement in *Macrolepiota* sect. *Macrolepiota*. *Macrolepiota capelariae* is characterized by its medium to large and very tall basidiomata, light brown to pale greyish brown, brownish orange or clay brown pileus surface, ellipsoid to oblong, dextrinoid basidiospores with a distinct germ pore, narrowly clavate to sometimes clavate cheilocystidia, a trichodermal pileus covering composed of mostly clavate to narrowly clavate, thick-walled terminal elements, and absence of clamp connections. Nomenclatural types are deposited at the herbarium SP from the 'Instituto de Pesquisas Ambientais', São Paulo, Brazil. A comparison with allied species is provided. An earlier varietal name proposed based on a Brazilian material, viz. *Lepiota procera* var. *vulpina* Rick, could corresponds to a synonym of the new taxa, but the lack of nomenclatural type, extant original herbarium specimens, and the record of confident distinctive characters for this variety prevent a reliable correlation with the new species proposed.

Keywords: nrITS, phylogeny, integrative taxonomy, biodiversity, Neotropics

Introduction

Macrolepiota Singer (1948:141) was proposed to include members of Agaricaceae with white to cream color spore print, clamped hyphae, and giant basidiospores with a metachromatic inner wall in cresyl blue. Later, Heinemann (1989), Singer (1986), and Vellinga *et al.* (2003) complemented the recognition of the genus, characterizing it by including members with relatively large and fleshy basidiomata; a stipe with a complex, mobile ring and a striking, banded appearance due to the presence of hymeni-trichodermal patches; lamellae free to remote; with relatively large and ellipsoid to amygdaloid-ellipsoid basidiospores, which have a rounded apex with a germ pore covered by a hyaline cap; pleurocystidia absent; a trichodermal pileus covering; and clamp connections present or more rarely absent.

The traditional infrageneric classification adopted by Singer (1986) recognized two sections based on the presence or absence of clamp connections, viz. *Macrolepiota* sect. *Macrolepiota* with clamp connections on the hyphae of the trama or stipe or at the base of the basidia; and *Macrolepiota* sect. *Macrosporae* (Singer) Bon (1979:40) without clamp connections on the trama or present only at the base of cheilocystidia. Later, Ge *et al.* (2010), based on molecular analyses with nuc rITS1-5.8-ITS2 (nrITS) sequences, proposed a new section, viz. *Macrolepiota* sect. *Volvatae* Z.W. Ge, Zhu L. Yang & Vellinga (Ge *et al.* 2010:97), to accommodate representatives with volva on the stipe base, small

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and amygdaliform-ellipsoid basidiospores, and no clamp connections. The authors also recovered in their analyses the clades corresponding to *Macrolepiota* sect. *Macrolepiota* and *Macrolepiota* sect. *Macrosporae*.

Macrolepiota, with a widespread distribution, can be found in various habitats, from grasslands to native forests, and even in man-made habitats, such as gardens, lawns, and compost-heaps (Vellinga *et al.* 2003, Sysouphanthong *et al.* 2021). It appears to be geographically structured, with closely related species in the same area, and no known widely distributed species (Vellinga 2003, Vellinga *et al.* 2003, Ge *et al.* 2010, Vizzini *et al.* 2011, Cho *et al.* 2019). For instance, *M. procera* (Scop.) Singer (1948:141), an assumed widespread species, is now known to only occurs in Europe and temperate Asia. Its occurrence outside the Eurasian continental area has not been confirmed (Vellinga 2003, Vellinga *et al.* 2003, Ge *et al.* 2010).

While *Macrolepiota* species from the Northern Hemisphere are well-understood, the scarce diversity data for Neotropical *Macrolepiota* has helped to perpetuate taxonomic confusion regarding species delimitation and distribution. This is in part due to the widespread use of species epithets typified by specimens from Europe [viz. *M. procera*, *M. mastoidea* (Fr.) Singer (1951:417), *M. excoriata* (Schaeff.) Wasser (1978:516), and *M. fuligineosquarrosa* Malençon (1979:261)] for Neotropical collections, and the overlapping suites of morphological traits in the basidiomata of species in this genus (Vellinga *et al.* 2003). The recent inclusion of molecular approaches to taxonomic studies revealed the uncertainty of previous morphological identifications of Neotropical *Macrolepiota* species (Fazolino Perez *et al.* 2018, Freitas & Menolli 2019), and it now becomes clear that those species need to be re-examined using molecular data and described as independent unique taxa.

A total of 15 species of *Macrolepiota* has been reported from five states in Brazil, but the occurrence of species described from Europe, Africa, Asia, and Australia still has to be confirmed by molecular methods, and is in fact not likely:

i) *M. bonaerensis* (Speg.) Singer (1951:417) from the states of Minas Gerais (Rosa & Capelari 2009), Paraná (Meijer 2006, 2008, Maki & Paccola-Meirelles 2002), Rio Grande do Sul [Rick 1907, 1908 as *Lepiota bonaerensis* (Speg.) Speg. (Saccardo 1887:28), 1939 as *Lepiota excoriata* f. *bonaerensis* (Speg.) Rick (1939:318), 1961 as *L. bonaerensis*, Singer 1954 as '*Lepiota procera* f. *bonaerensis* (Speg.) Rick', Guerrero & Homrich 1983, Putzke *et al.* 2014], and São Paulo (Spegazzini 1889 as *L. bonaerensis*, Pegler 1997);

ii) *M. brasiliensis* (Rick) Raithelh. (Raithelhuber 1988:64) from Rio Grande do Sul [Rick 1907, 1939, 1961 as *Lepiota permixta* var. *brasiliensis* Rick (1907:68), Raithelhuber, 1988, 1991];

iii) *M. colombiana* Franco-Mol. (Franco-Molano 1999:14) from Rio Grande do Sul (Putzke *et al.* 2014) and Paraná (Ferreira & Cortez 2012);

iv) *M. cyanolamellata* Fazolino, Lechner & Suaza Blandoìn (Fazolino Perez *et al.* 2018:934) from Rio Grande do Sul (Fazolino Perez *et al.* 2018);

v) *M. dolichaula* (Berk. & Broome) Pegler & R.W. Rayner (1969:365) from São Paulo (Grandi *et al.* 1984);

vi) *M. dunensis* D.S. Freitas & Menolli (2019:228) from Rio Grande do Norte (Freitas & Menolli 2019);

vii) *M. excoriata* from Rio Grande do Sul [Rick 1907, 1939, 1961 as *Lepiota excoriata* (Schaeff.) P. Kumm. (Kummer 1871:135), Raithelhuber 1988, 1991];

viii) *M. fuligineosquarrosa* from Rio Grande do Sul (Alves *et al.* 2016);

ix) *M. kerandi* (Speg.) Singer (1951:417) from Rio Grande do Sul (Putzke *et al.* 2014);

x) *M. mastoidea* from Minas Gerais (Rosa & Capelari 2009), Rio Grande do Sul [Rick 1939, 1961 as *Lepiota procera* f. *gracilenta* (Krombh.) Rick (1939:318), Raithelhuber 1988, 1991 as *M. gracilenta* var. *acuteumbonato* Raithelh. (1988:64), Putzke *et al.* 2014, Alves *et al.* 2016 as *M. gracilenta* (Krombh.) Wasser (1978:516)], and São Paulo (Grandi *et al.* 1984);

xi) *M. procera* from Rio Grande do Sul [Rick 1939 as *Lepiota procera* var. *vulpina* Rick (1939:317), Alves *et al.* 2016] and São Paulo (Bononi *et al.* 1984);

xii) *M. pulchella* de Meijer & Vellinga (Vellinga & Yang 2003:184) from Paraná [Heinemann & de Meijer 1996 as *Volvolepiota brunnea* (Rick) Singer (1959:12), Vellinga & Yang 2003, Meijer 2006], Rio Grande do Sul [Rick 1938, 1961 both as *Lepiotella brunnea* Rick (1938:251); Singer 1954, 1959 both as *V. brunnea*], and São Paulo (Bononi *et al.* 1981 as *V. brunnea*, Freitas & Menolli 2019);

xiii) *M. sabulosa* Fazolino & R.M. Silveira (Fazolino *et al.* 2018:936) from Rio Grande do Norte [Fazolino Perez *et al.* 2018, Freitas & Menolli 2019 as *M. sabulosa* var. *velistellaris* D.S. Freitas & Menolli (Freitas & Menolli 2019:234)];

xiv) *M. stercoraria* (Rick) Raithelh. (Raithelhuber 1988:63) from Rio Grande do Sul [Rick 1939, 1961 as *Lepiota stercoraria* Rick (1939:318), Raithelhuber 1988, 1991];

xv) *M. zeyheri* (Fr.) Singer (1962:67) from Rio Grande do Sul (Raithelhuber 1988, 1991).

Fazolino Perez *et al.* (2018) and Freitas & Menolli (2019) were the first authors to infer the phylogenetic positioning of Brazilian collections of *Macrolepiota* based on nrITS sequences of six species: *M. cyanolamellata* (Fazolino Perez *et al.* 2018), *M. dunensis* (Freitas & Menolli 2019), *M. sabulosa* (Fazolino Perez *et al.* 2018, including *M. sabulosa* var. *velistellaris* by Freitas & Menolli 2019), *M. pulchella* (Freitas & Menolli 2019, including the record of "*Macrolepiota* sp. 3" by Fazolino Perez *et al.* 2018), and the unnamed *Macrolepiota* sp. 1 and sp. 2 (Fazolino Perez *et al.* 2018).

Here, a new species of *Macrolepiota* is described from a fragment of the Atlantic Rainforest in São Paulo, Southeast Brazil. The new species was compared to previously described *Macrolepiota* species from the world literature, and their novelty demonstrated by comparison with morphologically similar described species and by molecular data (nrITS).

Materials and methods

Collection site

The collections of the new taxa have been found for many years in the 'Parque Estadual das Fontes do Ipiranga' (PEFI), a State Park that represents a remnant of the Atlantic Rainforest in São Paulo, Southeast Brazil. The forest is mainly composed of plant members of Myrtaceae, Fabaceae, Lauraceae, Melastomataceae, and typical genera such as *Eugenia*, *Myrcia*, *Ocotea*, and *Psychotria* (Barros *et al.* 2002). The PEFI is a large fragment of Atlantic Rainforest (526 ha) inserted in the urban area of the metropolitan region of São Paulo city, state of São Paulo, between 23º 38' 08" S–23º 40' 18" S and 46º 36' 48" W–46º 38' 00" W (Fernandes *et al.* 2002). The Park is at elevations between 770 and 825 m a.s.l (Nastri *et al.* 1992), with its predominant vegetation classified as Dense Ombrophilous Forest (Veloso *et al.* 1991), and a temperate climate with warm summers and dry winters (Cwa), according to the Köppen-Geiger climate classification system (Kottek *et al.* 2006, Peel *et al.* 2007).

Data from the Global Biodiversity Information Facility (GBIF), which covers data from iNaturalist (https://www.inaturalist.org.), and the Mushroom Observer (https://mushroomobserver.org) were consulted to access the geographical occurrence of specimens macromorphologically similar to the new species and which putatively represent the same taxon. Photos from the aforementioned platforms were downloaded to illustrate the species macromorphological identity from records outside the type locality. We followed the Creative Commons (https://creativecommons.org/) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CC BY, CC BY-NC, CC BY-SA, CC BY-ND, CC BY-NC-ND, and CC BY-NC-SA; for licenses and code attributions, please check https://creativecommons.org/licenses/?lang=en).

This study is according to the Brazilian legislation on access to biodiversity and is registered in the 'Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen #ABA9DB4).

Morphological methods, notation and use of standards

Macromorphological descriptions were based on field notes and color photographs of basidiomata taken in the field. Basidiomata were dried in a hot air dehydrator (45 °C). Color notation of fresh basidiomata is according to Küppers (2002), with color codes noted in parentheses (e.g., $N_{60}C_{60}Y_{50}$). Details of the basidiomata surface were examined under a Zeiss Stemi 305 stereo microscope.

Microscopic analyses were performed by a Zeiss Axioscope 5 microscope with bright field, phasecontrast optics and a charge-coupled device camera (Zeiss Axiocam 208 color), using sections of fresh or rehydrated tissues. Dried samples were rehydrated in distilled water or moistened in ethanol 70%, and microsections were cut by hand with a razor blade. Microscopic structures were examined in water, 3% KOH, Congo red, Cotton Blue, Cresyl Blue and Melzer's reagent, separately. At the beginning of a set of basidiospore data, the abbreviation [*a/b/c*] signifies "*a*" basidiospores measured from "*b*" basidiomata of "*c*" collections. Dimensions of basidiospores are presented in the following form *(m–) n–o (–p),* in which "*m*" is the smallest value observed or calculated and "*p*" is the largest value observed or calculated. In the range of values observed or calculated, the 5th percentile is "*n*"; and the 95th percentile is "*o*". A summary of definitions of biometric variables follows:

$\mathbf{L_m}$, ($\mathbf{W_m}$) = the average of all lengths (widths) of basidiospores measured.

Q = the ratio of length to width of a basidiospore or the range of such ratios for all basidiospores measured.

$\mathbf{Q_m}$ = the average of all Q values computed for all basidiospores measured.

The form of the basidiospores was interpreted based on the Q values following Bas (1969), and descriptive terms

for other morphological features follow Vellinga (1988) and Vellinga & Noordeloos (2001). The width of the basidia was taken at the widest part and the length was measured from the apex without sterigmata to the basal septum.

The collections examined including the selected nomenclatural types are deposited at the mycological collection of the herbarium SP from the 'Instituto de Pesquisas Ambientais', São Paulo, Brazil (Thiers, continuously updated).

Molecular methods and phylogenetic analyses

The procedure of extraction and amplification of DNA was based on the protocol of Binder & Hibbett (2003) from small parts of lyophilized material or directly from dried basidiomata fragments. The nrITS region was amplified using the primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993). Sequences of the nuclear large subunit (nLSU) were generated for the holotype (*DAZ060*) and two paratypes collections (*MC4584*, *MC4588*) using the primers LROR and LR5 (Vilgalys & Hester 1990, James *et al.* 2006), although they were not used in the phylogenetic analyses. The PCR products were cleaned with PureLink PCR Purification Kit (Invitrogen) according to the manufacturer's instructions and then sequenced using the same primer pairs.

Phylogenetic analyses were conducted with *Leucoagaricus leucothites* (Vittad.) Wasser (1977:308) and *Leucoagaricus nympharum* (Kalchbr.) Bon (1977:19) as outgroup and including sequences of *Macrolepiota* from GenBank based on sampling for recent phylogenetic analyses of Brazilian collections (Fazolino Perez *et al.* 2018, Freitas & Menolli 2019) with the additional sequences generated here (ON753529–ON753534). GenBank accession numbers for each collection are given in Fig. 1. The alignment was conducted using MAFFT 7.222 (Katoh & Standley 2013) with auto strategy and edited manually in Geneious 7.0.6 (Kearse *et al.*, 2012). The best nucleotide evolution model was estimated using the AIC criterion (Akaike Information Criterion) in PartitionFinder 2.1.1 (Lanfear *et al.* 2012, 2016, Guindon *et al.* 2010). The nrITS region was partitioned into ITS1, 5.8S and ITS2, and the evolution model was estimated for each partition. A Maximum Likelihood (ML) analysis was performed in RAxML 8.2.12 (Stamatakis 2014) in the CIPRES Science Gateway 3.3 (Miller *et al.* 2010) using the GTR+GAMMA model with a rapid bootstrap analysis with 1,000 replicates and search for the best-scoring ML tree. The Bayesian inference (BI) analysis was performed with MrBayes v3.2.7a (Ronquist *et al.* 2012) in the CIPRES Science Gateway 3.3 (Miller *et al.* 2010) using three partitions (ITS1, 5.8S, ITS2), with TVM+G model for ITS1 and ITS2, and JC model for 5.8S, with two independent runs, four simultaneous independent chains and 10,000,000 generations with a sample frequency every 5,000 generation. The following abbreviations are used for statistical data: Bootstrap (BS) and Posterior Probability (PP).

Results

Phylogeny

The nrITS amplification products of the analyzed samples ranged from 400–644 bp. A total of 94 sequences were used for the nrITS phylogeny, including the six newly sequenced collections and the outgroups. The multiple sequence alignment was 723 characters long in total.

Both ML and BI analyses resulted in similar tree topologies and so only the ML tree is illustrated (Fig. 1) with both support values shown near the clade nodes. It was verified, with the highest support (100% BS, 1 PP), the monophyly of the genus, which is organized in three main clades corresponding to the sections indicated by Ge *et al.* (2010): *Macrolepiota* sect. *Macrolepiota* (unsupported), *Macrolepiota* sect. *Macrosporae* (89% BS, 1 PP), and *Macrolepiota* sect. *Volvatae* (100% BS, 1 PP).

All sequences generated here from the new species formed a well-supported clade (97% BS, 1 PP) within the clade corresponding to *Macrolepiota* sect. *Macrolepiota* and together with two sequences (MN847716 and MH290361) of two unnamed collections from Argentina and Mexico that most likely also represent *M. capelariae*. All sequences in *M. capelariae* clade are 98.78–100% identical, with MN847716 (Argentina) and MH290361 (Mexico) presenting, respectively, levels of 0.31% and 0.93% sequence divergence compared to the holotype of *M. capelariae*. *Macrolepiota capelariae* is related (86% BS, 1 PP) to three other *Macrolepiota* species, viz. *M. colombiana* from Colombia and two clades corresponding to species of unnamed collections from Argentina and Brazil (Fig. 1).

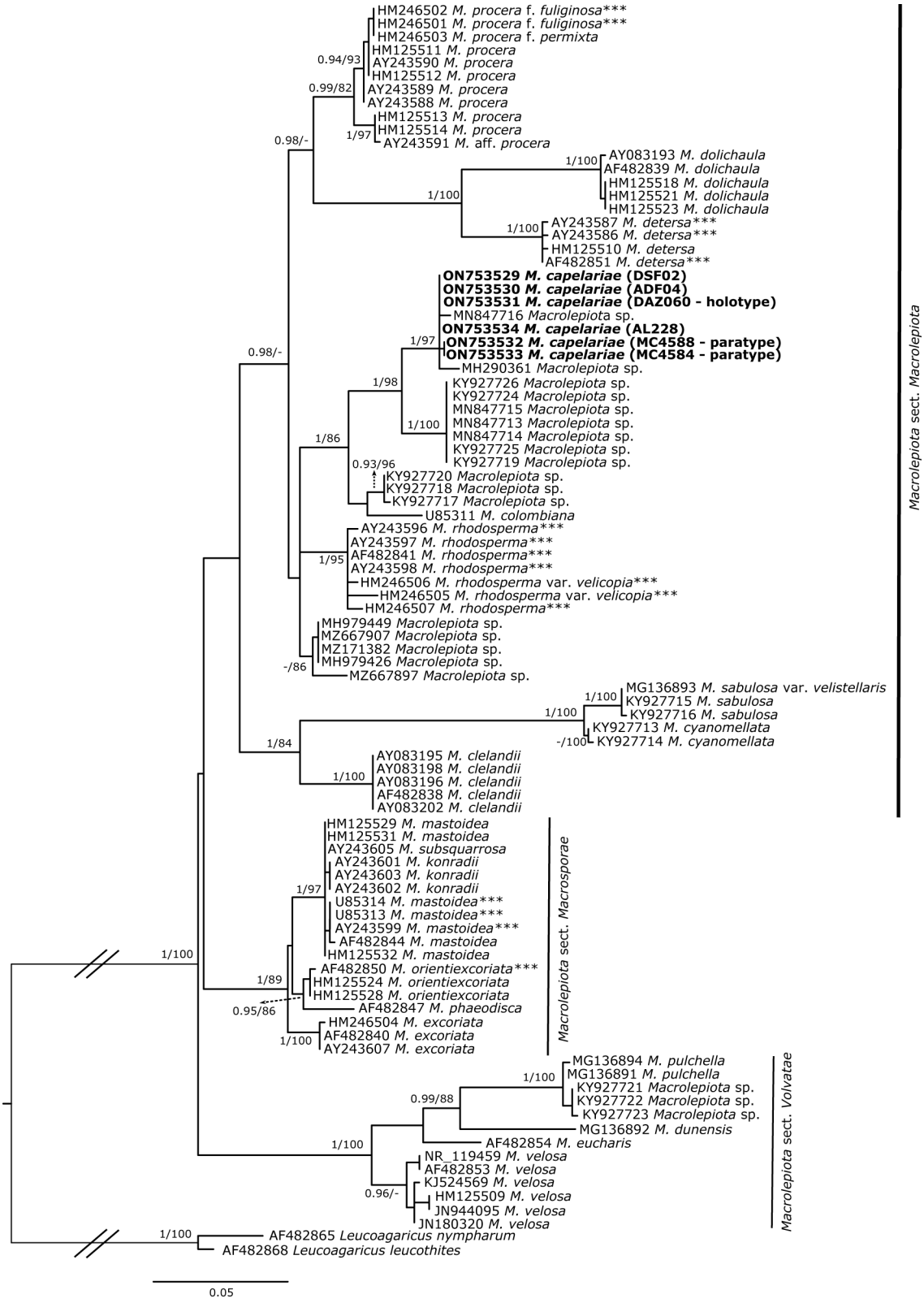

FIGURE 1. Best tree from the ML analysis of *Macrolepiota* based on nrITS data and rooted with *Leucoagaricus leucothites* and *L. nympharum*. The sequences generated in this work are in bold. Support values (BS >70% and PP >0.90) are shown near the node branches. Root length has been reduced to facilitate graphical representation. A dash (-) indicates that the support values for the clade are below those indicated above. *** indicates taxon names modified according to Vizzini *et al.* (2011).

Taxonomy

Macrolepiota capelariae A.D. Souza, C.C. Nascimento & Menolli *sp. nov.* (Figs. 2–4)
MycoBank: MB 844529

Diagnosis:—Similar to *Macrolepiota colombiana* but differing in brownish orange or clay brown, light brown to pale greyish brown pileus surface, mostly breaking up into radially arranged interwoven strips, and then exposing a whitish background, the less complex annulus, the unchanging context, the non-septate cheilocystidia, and absence of clamp connections.

Etymology:—'*capelariae*', in honor of Dr. Marina Capelari, a dedicated Brazilian mycologist for her contribution to the taxonomic study of mushrooms from Brazil for more than 30 years during her career.

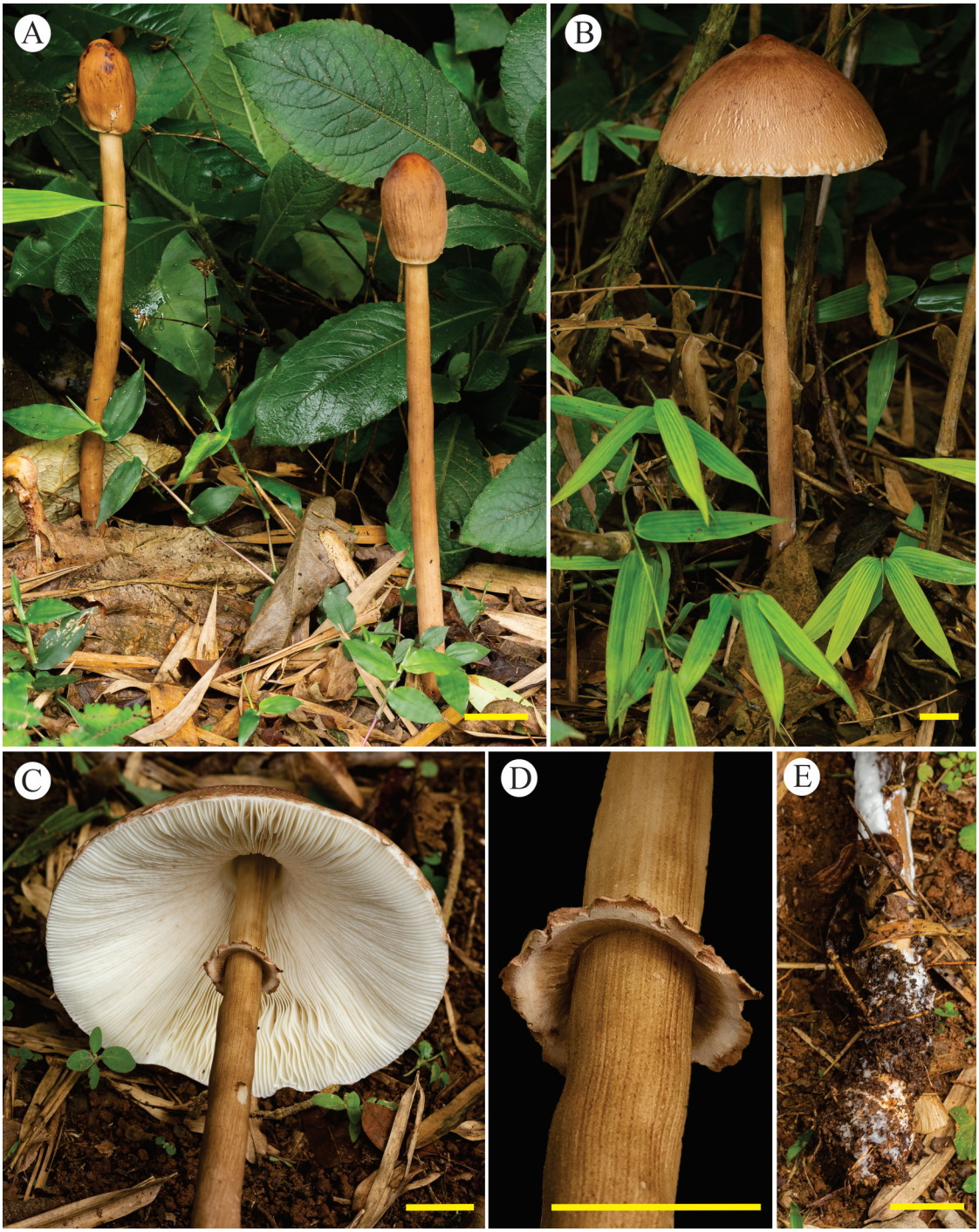

FIGURE 2. *Macrolepiota capelariae* (SP513052, holotype). **A.** Young basidiomata. **B.** Mature basidioma. **C.** Detail of the lamellae. **D.** Detail of the annulus. **E.** Detail of the stipe base. Scale bars = 20 mm. Photos by D.A. Zabin.

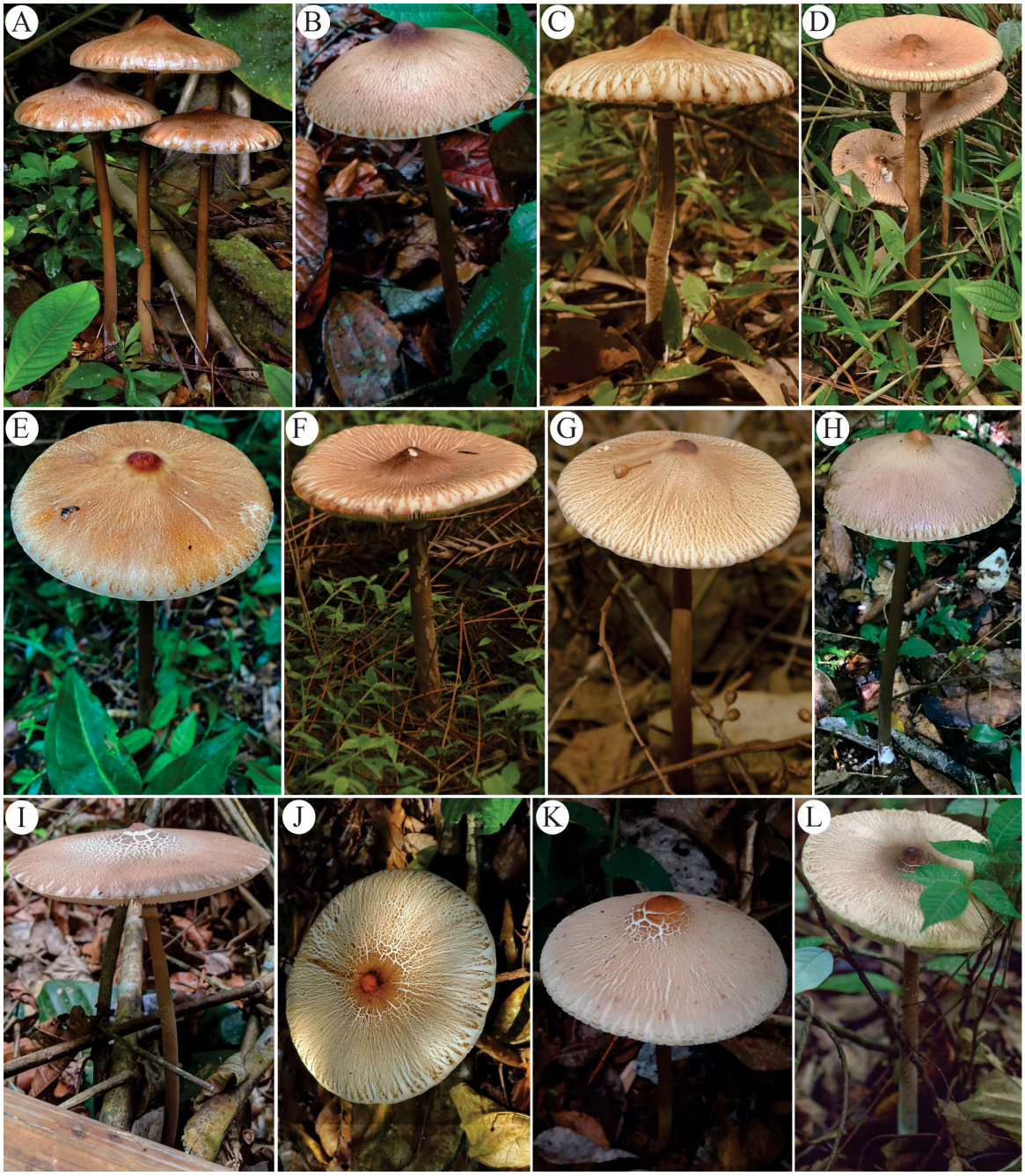

FIGURE 3. Putative records of *Macrolepiota capelariae* outside the type locality. Data from GBIF/iNaturalist and Mushroom Observer. **A**–**K.** Brazil (**A**, **J**, **K**: Rio de Janeiro, RJ; **B**: Rio Branco, AC; **C**: Lajeado, RS; **D**: Jaraguá do Sul, SC; **E**: Florianópolis, SC; **F**: Palmeira, PR; **G**: São Sebastião da Grama, SP; **H**: Vitorino, PR; **I**: Senador Guiomard, AC). **L.** Mexico (Tepoztlán, Morelos). **Brazilian states abbreviations**: AC = Acre, PR = Paraná, RJ = Rio de Janeiro, RS = Rio Grande do Sul, SC = Santa Catarina, SP = São Paulo. **Photo credits**: **A.** ©Rogerio Dias, **B.** ©Gabriel Fernando, **C.** ©Edith Mello, **D.** ©fernandotorres1978, **E.** ©Mariana Lopes, **F.** ©andersonwarkentin, **G.** ©Café Gato-Mourisco, **H.** ©Alan Lucas Hentz Policarpo, **I.** ©Mayk Oliveira, **J.** ©rogerriodias, **K.** ©Alessandro Valente Vieira, **L.** ©Alejandro Tux.

Holotype:—BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 14 January 2022, *D.A. Zabin & M.C.S. Pires DAZ060* (SP513052), GenBank [nrITS]: ON753531, [nLSU]: ON794497.

Paratypes:—BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 24 July 2010, *M. Capelari, P.O. Ventura & N. Menolli Jr. MC4584* (SP513053), GenBank [nrITS]: ON753533, [nLSU]: OP302841; *ibid.* 06 December 2010, *M. Capelari MC4588* (SP513054), GenBank [nrITS]: ON753532, [nLSU]: OP302842; *ibid.* 20 March 2015, *D.S. Freitas DSF02* (SP513055), GenBank [nrITS]: ON753529; *ibid.* 800 m a.s.l, 26 February 2016, *A.D. Souza & L.S. Ramos ADF04* (SP513056), GenBank [nrITS]: ON753530; *ibid.* 03 March 2016, *A.A. Alcântara AL228* (SP513057), GenBank [nrITS]: ON753534.

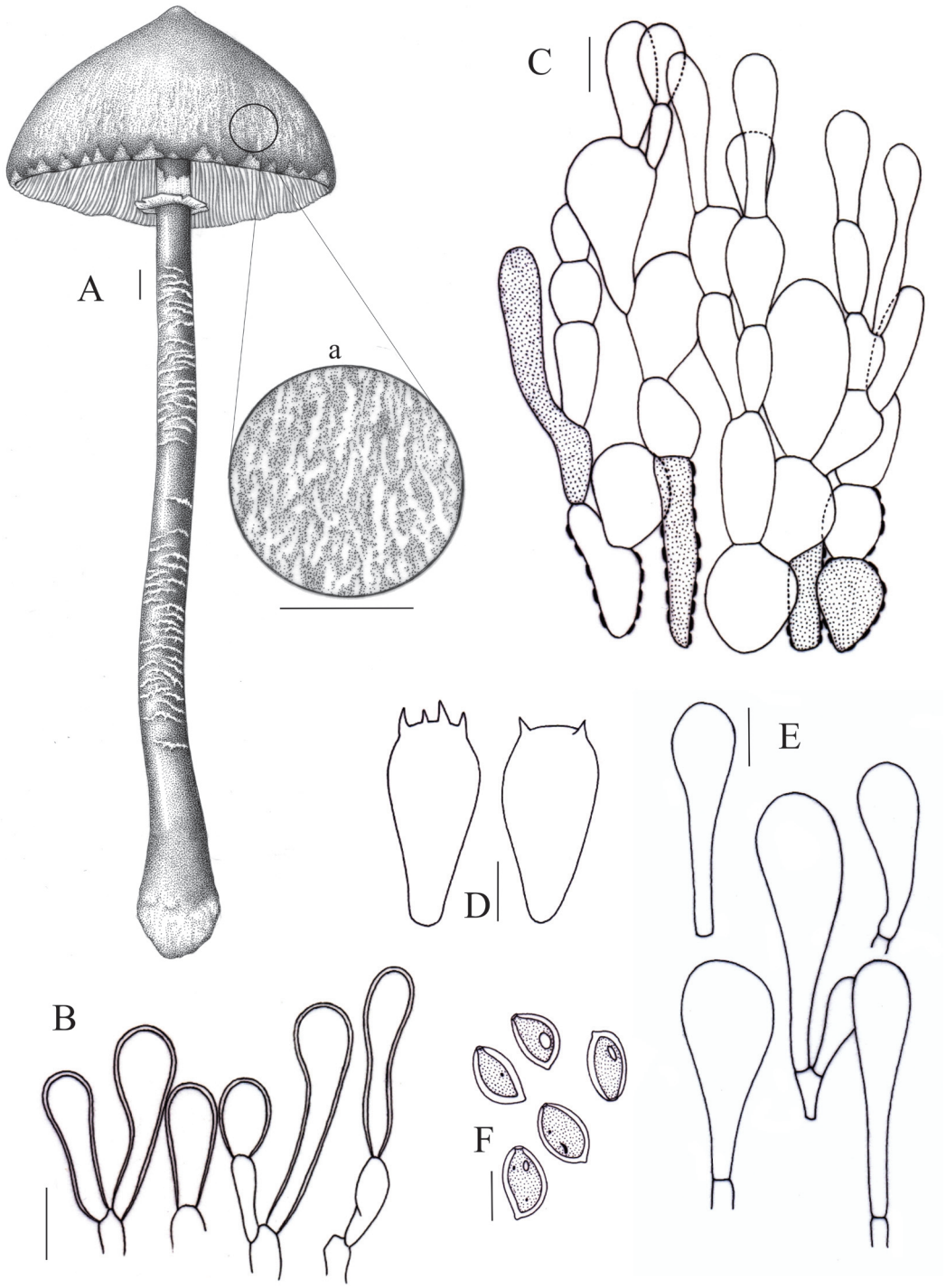

FIGURE 4. *Macrolepiota capelariae* (SP513052, holotype). **A**. Mature basidioma, **a**. Detail of the pileus surface. **B**. Apical thick-walled elements of the pileus covering. **C**. Pileus covering. **D**. Basidia. **E**. Cheilocystidia. **F.** Basidiospores. Scale bars: A/a = 10 mm; B–F = 10 µm. Drawings: A/a by K. Sousa, B–F original by C.C. Nascimento and inked by K. Sousa.

Description:—*Basidiomata* medium to large-sized. *Pileus* 78–120 mm diam., fleshy, ovoid to hemispherical when young, expanding to broadly campanulate, convex, rounded-umbonate, obtuse umbonate to plano-umbonate, sometimes applanate with a slightly reflexed margin with age; surface dry, at first brownish yellow ($N_{20}Y_{70\text{-}90}M_{30}$) to brownish orange ($N_{20}Y_{70\text{-}90}M_{30}$), then light brown ($Y_{30\text{-}40}M_{30}C_{20}$, $Y_{40}M_{30\text{-}40}C_{30}$) to pale greyish brown ($N_{40}Y_{30\text{-}50}M_{20}$), brownish orange ($Y_{60}M_{30\text{-}40}C_{10\text{-}20}$) or clay brown ($Y_{40}M_{50}C_{20}$), breaking up into radially arranged interwoven strips, but sometimes into small to large patch-like squamules (easily detachable from the pileus), and then exposing a whitish background; disc smooth, dark brown ($N_{80}A_{90}M_{50}$–$N_{90}A_{20}M_{60}$) to reddish brown ($N_{50}Y_{80}M_{50}$–$N_{60}Y_{80}M_{70}$). *Lamellae* free,

remote from the stipe, crowded, ventricose, white when young, white to beige ($Y_{20}M_{10}C_{10}$) when mature, sometimes with brownish spots; lamellulae attenuate, in 1–2 ranks, unevenly distributed; edge entire, smooth, concolorous. *Stipe* 150–320 × 5–18 mm, central, subcylindrical, gradually attenuating upwards, bulbous at base; bulb subglobose, 20–25 mm wide, completely covered by a tomentose-velvety whitish mycelial layer, often with long white rhizomorphs; surface medium brown ($N_{50}Y_{60}M_{30}$–$N_{60}Y_{40\text{-}50}M_{40}$), paler upwards, finely fibrillose, sometimes breaking open into pale brown ($Y_{60}M_{50}C_{50}$) zigzagging bands over the middle zone on an off-white background. *Annulus* superior, membranous, beige ($N_{40}A_{10}M_{10}$) at the upper side, pale brown at underside, with a broken brownish margin, movable when mature. *Context* in the pileus white and moderately thick, in stipe white and hollow with a central white cottony strand, unchanging in both. *Odor* fungoid. *Taste* mild, sweet. *Spore print* white.

Basidiospores [255/16/16] (8.8–)12.5–15.0(–17.5) × (5.8–)7.5–11.2(–11.8) µm ($\mathbf{L_m}$= 13 µm; $\mathbf{W_m}$ = 8.5 µm; Q = 1.3–2.0; $\mathbf{Q_m}$ = 1.54), ellipsoid to elongate, with germ pore covered by hyaline cap, smooth, hyaline, thick-walled, dextrinoid, congophilous, metachromatic in Cresyl blue; apiculus about 1 µm long. *Basidia* 22–42 × 11.2–15.0(–16.2) µm, clavate, hyaline, thin-walled, 1–4-spored. *Lamella edge* sterile, with crowded cheilocystidia. *Cheilocystidia* 21–50 × 9.0–15.0(–16.5) µm, narrowly clavate, sometimes clavate, often catenulate, hyaline, colorless, thin-walled. *Pleurocystidia* absent. *Pileus covering* a trichoderm composed of 4–6 layers of elements, often branching; lower layer elements subglobose, broadly clavate to oblong, seldom cylindrical, 18.0–32 × 11.0–25 µm, slightly thick-walled to thick-walled, with pale yellowish brown intracellular pigments, walls sometimes encrusted; upper layer elements mostly clavate to narrowly clavate, 11.5–32 × 3.0–11 µm, slightly thick-walled to thick-walled, with hyaline to pale yellowish brown parietal pigment. *Stipe covering* a hymeniderm of cylindrical elements, 6.2–14.7 µm wide, thin-walled, with intracellular pale brown content. *Clamp connections* absent.

Habitat and distribution:—Saprotrophic and terrestrial, growing in a remnant of Atlantic Rainforest, under shade trees, solitary or in small groups, known from the state of São Paulo, Southeastern Brazil (type locality). Based on records from GBIF, Mushroom Observer, and GenBank (MN847716 and MH290361), the species distribution can be extended to Argentina and Mexico, and putatively to five other Brazilian states: Acre, Paraná, Rio de Janeiro, Rio Grande do Sul, and Santa Catarina.

Additional specimens examined:—Brazil, SP, São Paulo, Parque Estadual das Fontes do Ipiranga (PEFI), 800 m a.s.l., 14 October 1998*, L. Gusmão s.n.* (SP513059); *ibid.* 15 October 1998*, M.Capelari s.n.* (SP513060); *ibid.* 10 October 2001, *U.C. Peixoto s.n.* (SP381602); *ibid.* 26 December 2001, *U.C. Peixoto s.n.* (SP381601); *ibid.* 05 September 2002*, U.C. Peixoto s.n.* (SP381603); *ibid.* 11 December 2003, *M.D.F. Trude LJG 022/03* (SP381600); *ibid.* 30 January 2008*, T.V.S. Campacci s.n.* (SP513061); *ibid.* 04 April 2012, *M. Capelari MC4692* (SP513062); *ibid.* 26 February 2016, *A.D. Souza ADF03* (SP513058).

Discussion

Among the taxa in *Macrolepiota* sect. *Macrolepiota* not sampled in the phylogenetic tree, the following must be considered to support the morphological differences and the proposition of novelty for *M. capelariae*: (1) *Lepiota procera* var. *vulpina*, (2) *M. bonaerensis*, (3) *M. africana* (R. Heim) Heinem. (Heinemann 1969:207), (4) *M. zeyheri*, (5) *M. brasiliensis*, and (6) *M. stercoraria*. Additionally, *M. colombiana*, which is sampled in our phylogenetic analyses based on authentic material from Colombia (Franco-Molano, 1999), is also compared to *M. capelariae*, although our nrITS tree (Fig. 1) clearly supports the molecular distinction of both species. Furthermore, based on the pictures, collecting area and/or morphological descriptions presented by Bononi *et al.* (1984), Grandi *et al.* (1984), Ferreira and Cortez (2012), and Alves *et al.* (2016), the records of *M. procera*, *M. colombiana*, and *M. mastoidea* by these authors most likely also represent *M. capelariae*, although a careful re-examination of the samples studied by them is necessary to confirm this.

Lepiota procera var. *vulpina* comes quite close to *M. capelariae*. However, it was described by Rick (1939) without any holotype indication and based on a pretty short Latin protologue: "colore vulpine-fulvo et squamis innatis. Color Lep. procerae generatium est griseo-brunneus". Although the color and ornamentation of the pileus as described by Rick (1939) resemble those of *M. capelariae*, the lack of extant original herbarium specimens and record of any other distinctive character for *L. procera* var. *vulpina* prevent a reliable correlation with the new species proposed here. Nevertheless, the characters described by Rick (1939) for a supposed typical variety of *L. procera* (= *M. procera*) from Brazil are distinct from those recorded to *M. capelariae*, such as the larger pileus (up to 30 cm wide) and the longer basidiospores (14–22 µm). Rick (1961) also listed *Lepiota gigantea* Speg. (Spegazzini 1909:260) as a

synonym of *L. procera* var. *vulpina*, but still the first's original description does not resemble *M. capelariae* in some recorded characters. *Lepiota gigantea*, known from Argentina, differs from *M. capelariae* in its much larger size, with a maximum reported pileus diameter of 25 cm and stipe length/width of 90 × 3 cm, and by a distinctly acute umbonate pileus with a striate margin; no data is provided for the basidiospores (Spegazzini 1909). A study with a thorough taxonomic review of the *Macrolepiota* species described from the Neotropics, and all relevant Rick's names, could help to verify if *Lepiota procera* var. *vulpina* should be listed as synonym of *M. capelariae*, but no collection under this varietal name were located at PACA herbarium (curator pers. comm.) neither at the database (https://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm) of the 'Lloyd Herbarium' collections that are now part of the U.S. National Fungus Collections (BPI), both where the Rick's collections are deposited.

Macrolepiota bonaerensis, described as *Agaricus bonaerensis* Speg. (Spegazzini 1880:278) from Argentina and then combined in *Macrolepiota* by Singer (1951:417), differs from *M. capelariae* in its smaller basidiomata (pileus 50–90 mm diam., stipe 80–10 × 5–10 mm), conspicuous whitish pileus background, fimbriate to appendiculate pileus margin, larger basidiospores (14.5–16 × 11.3–11.8 µm), and clamped hyphae in the stipe context (Spegazzini 1880, Singer 1951, Singer & Digilio 1952). *Macrolepiota bonaerensis* was reported from grassy habitats, always outside woodland, and in a totally different habitat than that of *M. capelariae* (Spegazzini 1880, Singer 1951, Singer & Digilio 1952).

When compared to *M. capelariae*, *M. africana*, originally described from Central African Republic and Cameroon (Heim 1968:212 as *Leucocoprinus africanus*) but later reported from other parts of Africa (Heinemann 1969, Pegler 1977, Härkönen *et al.* 2003), has a larger pileus [100–150(–300) mm] with radially tomentose dark brown surface, a more complex annulus with brown squamules on the lower surface, slightly smaller basidiospores [12.2–13.9(–15) × 7.6–9.9 µm], and clamped hyphae in the context (Heinemann 1969, Pegler 1977). Moreover, the trichodermal layer of *M. africana* is composed of cylindrical, septate hyphae that seldom branch, with some terminal elements (40–120 × 6–13 µm) developing a thick brown wall and an acutely pointed apex (Heinemann 1969; Pegler 1977). In *M. capelariae*, the trichodermal layer is formed of slightly thick-walled to thick-walled terminal elements with an obtusely rounded apex, not exceeding 32 µm long.

Macrolepiota zeyheri, originally described from South Africa as *Agaricus zeyheri* Fr. (Fries 1848:122), can be distinguished from *M. capelariae* by the appendiculate pileus margin, the whitish cream to pinkish lamellae, stipe length [60–80(–100) mm] that rarely exceeds the pileal diameter (50–100 mm), and slightly smaller basidiospores, 11.5–15 × 7.5–10.5 µm (Pearson 1950, Heinemann 1969, Reid 1975, Pegler 1982, Raithelhuber 1988, 1991).

Macrolepiota brasiliensis and *M. stercoraria* were originally described from Southern Brazil as *Lepiota permixta* var. *brasiliensis* (Rick 1907:68) and *L. stercoraria* (Rick 1939:318), respectively, although with very short protologues. *Macrolepiota stercoraria* was described in more detail by Raithelhuber (1988). *Macrolepiota brasiliensis* and *M. stercoraria* are both of considerably smaller stature than *M. capelariae*, with a maximum reported stipe length of 120 mm, which does not reach the smallest stipe size observed for *M. capelariae*. In addition, *M. brasiliensis* differs from *M. capelariae* in having a whitish pileus, pale green tinge on lamellae, and smaller basidiospores (9.5–12.2 × 6–7 µm); whilst *M. stercoraria* is distinguished from *M. capelariae* in its brownish to ochraceous pileus squamules that are concentrically arranged on pileus surface, a depressed pileus center, and a yellowish annulus (Rick 1907, 1939, 1961, Raithelhuber 1988, 1991). Additionally, whether these taxa indeed belong to *Macrolepiota* and not to *Chlorophyllum* Masse (1898:135) still needs to be shown.

Finally, *M. colombiana*, originally described from Colombia in *Quercus humboldtii* Bonpl. (Humboldt & Bonpland 1809:155) and Pinaceae forests, is also characterized by robust basidiomata but differs from *M. capelariae* in phylogenetic placement (Fig. 1) and its dark brown central calotte at mature pileus that has large to small scales on a white background, more complex annulus, mostly septate and often branched cheilocystidia, and abundant clamp connections on stipe hyphae (Franco-Molano, 1999). Furthermore, *M. colombiana* has a white context slowly turning grayish-red when exposed and a very strong odor of cabbage (Franco-Molano, 1999).

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ANEXO B

MYCOLOGIA
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Unroughing the cat's tongue mushrooms: Four new species of *Pseudohydnum* from Brazil based on morphological and molecular phylogenetic evidence

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ABSTRACT

Pseudohydnum, commonly known as cat's tongue mushrooms, is a monophyletic assemblage within Auriculariales, which encompasses species with gelatinous basidiomata, spathulate, flabellate, or shell-shaped pileus, hydnoid hymenophore, globose to ellipsoidal basidiospores, and longitudinally cruciate-septate basidia. According to the available literature, 16 species have been described in *Pseudohydnum*, mostly represented in temperate-boreal forests of the Northern Hemisphere. However, the limited morphological, molecular, and ecological information, especially from the Southern Hemisphere ecosystems, does not presently allow a reliable assessment of its taxonomic boundaries nor provide a complete picture of the species diversity in the genus. In an ongoing effort to examine specimens collected in dense and mixed ombrophilous forest fragments (Atlantic Rainforest domain) from Southeastern and Southern Brazil, additional taxa assigned to *Pseudohydnum* were identified. Four new species are recognized based mostly on characters of the pileus surface, stipe, hymenium, and basidiospores. Molecular phylogenetic analyses based on nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS barcode), partial nuc rDNA 28S, and partial RNA polymerase II largest subunit (*RPB1*) sequences supported the description of these new taxa. Here, we propose *Pseudohydnum brasiliense, P. brunneovelutinum, P. cupulisnymphae*, and *P. viridimontanum* as new species. Morphological descriptions, line drawings, habitat photos, and comparisons with closely related taxa are provided. A dichotomous key for identification of currently known Southern Hemisphere *Pseudohydnum* species is presented.

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INTRODUCTION

Pseudohydnum P. Karst. is an enigmatic group of jelly fungi characterized by the stipitate to sessile gelatinous basidiomata, flabellate or shell-shaped pileus, hymenophore with sparse to crowded spines, globose to ellipsoidal basidiospores, and longitudinally cruciate-septate basidia (Bessette et al. 1996; Karsten 1868; Niveiro and Popoff 2011). The genus was originally introduced by Karsten (1868) within the order Tremellales because of these fungi's globose to ellipsoid basidia with longitudinal septa (Breitenbach and Kränzlin 1986) and later assigned to Auriculariales (Aporpiaceae) by Bandoni (1984), who redefined this order to accommodate heterobasidiomycetes with transversely septate basidia (auricularioid-type) as well as longitudinally septate basidia. This latter placement has been confirmed by some recent molecular phylogenetic studies, although *Pseudohydnum* has not yet been classified with certainty into a family (Chen et al. 2020; Spirin et al. 2023; Yuan et al. 2018).

Currently, only 16 species of *Pseudohydnum* have been described: (i) *Pseudohydnum abietinum* H.M. Zhou & Jing Si from northwestern China (Gansu Province) (Zhou et al. 2023), (ii) *P. alienum* Spirin & V. Malysheva from Russia (Karachay-Cherkessia, North Caucasus Region) (Spirin et al. 2023), (iii) *P. brunneiceps*

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Y.L. Chen, M.S. Su & L.P. Zhang from East China (Jiangxi Province) (Chen et al. 2020), (iv) *P. cystidiatum* V. Malysheva & V. Dudka from northwestern Vietnam (Cao Bằng Province) (Spirin et al. 2023), (v) *P. gelatinosum* (Scop.) P. Karst from western Slovenia (Inner Carniola) (Karsten 1868), (vi) *P. himalayanum* Y.C. Dai, F. Wu & H.M. Zhou from southwestern China (Yunnan Province) (Zhou et al. 2022), (vii) *P. meridianum* V. Malysheva & Spirin from central Vietnam (Gia Lai Province) (Spirin et al. 2023), (viii) *P. omnipavum* Spirin & Miettinen from western United States (Idaho state), (ix) *P. orbiculare* J.A. Cooper from New Zealand South Island (West Coast Region) (Zhou et al. 2022), (x) *P. placibile* V. Malysheva & V. Dudka from northwestern Vietnam (Cao Bằng Province) (Spirin et al. 2023), (xi) *P. sinobisporum* T. Bau, H.M. Zhou & Jing Si from northwestern China (Jilin Province) (Zhou et al. 2023), (xii) *P. sinogelatinosum* Y.C. Dai, F. Wu & H. M. Zhou from southwestern China (Yunnan Province) (Zhou et al. 2022), (xiii) *P. tasmanicum* Y.C. Dai & G.M. Gates from Tasmania, Australia (Zhou et al. 2022), (xiv) *P. totarae* (Lloyd) J.A. Cooper from New Zealand North Island (Wellington Region) (Zhou et al. 2022), (xv) *P. translucens* Lloyd from southern-central Japan (Lloyd 1925), and (xvi) *P. umbrosum* V. Malysheva & Spirin from Siberia, Russian Far East (Spirin et al. 2023). In addition, one subspecies, two forms, and two varieties of *P. gelatinosum* are recognized: *P. gelatinosum* ssp. *pusillum* (Ellis & Everh.) Miettinen & Viner from northwestern United States (Olympic Peninsula) (Spirin et al. 2023), *P. gelatinosum* f. *album* (Bres.) Kobayasi from Japan (Bresadola 1932; Kobayasi 1954), *P. gelatinosum* f. *fuscum* (Bres.) from Japan (Bresadola 1932; Kobayasi 1954), *P. gelatinosum* var. *bisporum* Lowy & Courtec. from French Guiana (Saut Pararé) (Courtecuisse & Lowy), and *P. gelatinosum* var. *paucidentatum* Lowy from western Bolivia (Nor Yungas Province) (Lowy 1959).

The type species of the genus, *P. gelatinosum*, commonly known as cat's tongue, false hedgehog mushroom, or white jelly mushroom, is considered edible and also a source of medicinal compounds, exhibiting anticancer, antibacterial, and antioxidant properties (Binion et al. 2008; Boulet 2003; Li et al. 2021; Sternisa et al. 2022; Wu et al. 2019). Although it was originally described from Europe, there are further regional treatments and reports, including North America (Volk et al. 1994), South America (Courtecuisse and Lowy 1990; Lowy 1971; Nivero and Popoff 2011), Central America (Lowy 1971; Mata et al. 2003), Asia (Dai 2010; Wu et al. 2009), and Oceania (McNabb 1964). Nevertheless, recent phylogenetic and diversity studies indicated that *P. gelatinosum* seems to be distributed only in temperate-boreal forests of the Northern Hemisphere, and its occurrence outside these areas is highly doubtful (Spirin et al. 2023; Zhou et al. 2022).

Despite some recent studies that employed molecular tools to unveil overlooked *Pseudohydnum* species (Chen et al. 2020; Spirin et al. 2023; Zhou et al. 2023, 2022), our knowledge of the genus' diversity and distribution is still incipient, especially in tropical and subtropical Southern Hemisphere forests, where extensive taxon sampling and molecular phylogenetic assessment of ambiguous collections are nonetheless lacking. Indeed, Brazilian taxonomic treatments of the genus are completely absent, except for a few reports of "*P. gelatinosum*" from the Amazon Forest (Bastos et al. 2015; Bononi 1981) and the description of *P. guepinioides* Rick from the Atlantic Rainforest (Rick 1904). The latter's protolog immediately evokes the concept of a stipitate *Trechispora* P. Karst species because of the cespitose, multilobate, cartilaginous-gelatinous basidiomata, and the ornamented spores (Rick 1904, 409); thus, Rick's taxon is considered a synonym of *T. thelephora* (Lév.) Ryvarden (Ryvarden 2002). The reports of "*P. gelatinosum*" from the Amazon Forest do not fit into any of the hitherto known species in the genus, likely referring to species yet to be described.

During our ongoing research on macrofungi focused on edible species, several specimens of *Pseudohydnum* were collected in Atlantic Rainforest fragments from Southern and Southeastern Brazil. The morphological examination and the molecular phylogenetic analyses for these collections revealed that they represent four new species. These species are formally described here as *P. brasiliense, P. brunneovelutinum, P. cupulisnymphae*, and *P. viridimontanum*. We accordingly present a morphological description of the new species and also provide a dichotomous key for identification of currently known Southern Hemisphere *Pseudohydnum* species.

MATERIALS AND METHODS

Collection sites.—Sixteen specimens were collected from December 2019 to February 2023 in the Brazilian states of Espírito Santo, Paraná, Rio de Janeiro, and São Paulo. Collecting areas include the following Atlantic Rainforest protected reserves: Parque Estadual Campos do Jordão (PECJ), Parque Estadual da Cantareira (PEC), Parque Estadual Ilha do Cardoso (PEIC), Parque Estadual Pico do Marumbi (PEPM), Parque Estadual Turístico do Alto Ribeira (PETAR), Parque Nacional da Serra da Bocaina (PNSB), Parque Nacional da Serra dos Órgãos

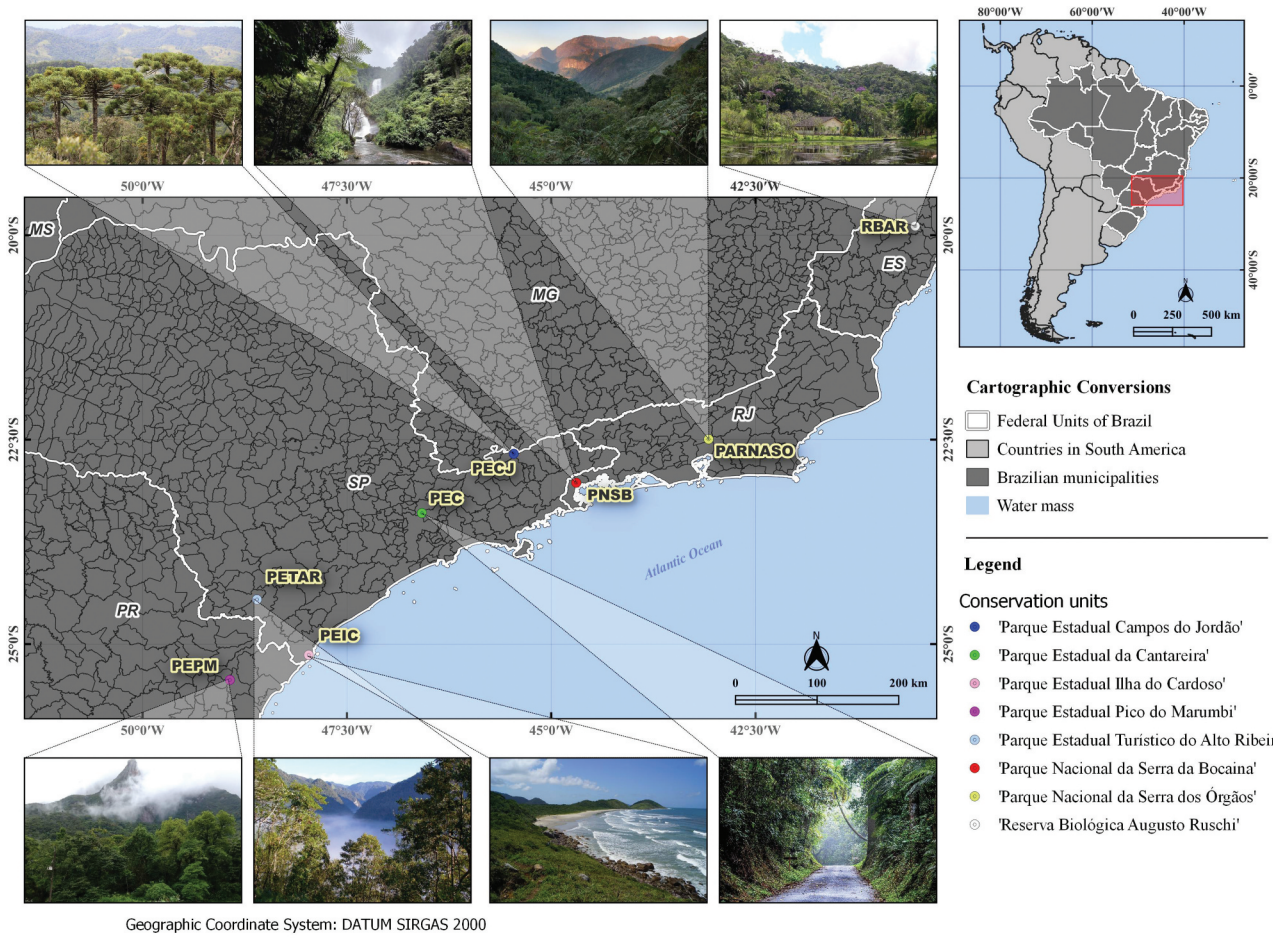

Figure 1. Geographic location of the sampling sites indicated as different color dots on the map with general vegetation aspects in the Brazilian states of Espírito Santo (ES), Rio de Janeiro (RJ), São Paulo (SP), and Paraná (PR).

(PARNASO), and Reserva Biológica Augusto Ruschi (RBAR) (FIG. 1). Additional specimens from the states of Minas Gerais, Rio Grande do Sul, Rondônia, São Paulo, and Santa Catarina were obtained from the herbaria SP [Herbarium of the Instituto de Pesquisas Ambientais (IPA)] and FLOR [Fungarium of the Universidade Federal de Santa Catarina (UFSC)] and from two private individuals, with consent.

The Brazilian Atlantic Rainforest hosts one of the world's most diverse tropical forest biota, supporting one of the highest degrees of species richness and rates of endemism on the planet (Mittermeier et al. 2004; Myers et al. 2000; Ribeiro et al. 2011). It is heterogeneous and composed of many different forest formations (dense ombrophilous, open ombrophilous, mixed ombrophilous, semideciduous seasonal, and deciduous seasonal) and a diversity of climates (tropical humid, subtropical of altitude, subtropical dry winter, and temperate) (Ab'Sáber 2003; Joly et al. 2014), with the annual average temperature ranging from 11.3 to 27.9 C, annual precipitation ranging from 643 to 3525 mm, and elevations from sea level to 2700 m above sea level (a.s.l.) (Joly et al. 2014; Oliveira-Filho 2017).

Data from the Global Biodiversity Information Facility (GBIF), which covers data from iNaturalist (https://www.inaturalist.org.https://mushroomobserver.org), were consulted to access the geographic occurrence of *Pseudohydnum* specimens from the American continent. Photos from the aforementioned platforms were downloaded to illustrate the macromorphological diversity of putatively undescribed *Pseudohydnum* species. We followed the Creative Commons (https://creativecommons.org/) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CCBY, CCBYNC, CCBYSA, CCBYND, CCBYNCND, and CCBYNCSA; for licenses and code attributions, see: https://creativecommons.org/licenses/?lang=en).

This study is in accordance with the Brazilian legislation on access to biodiversity and is registered in the "Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado" (SisGen no. A6F2384).

Morphological methods, notation, and use of standards.—To assess the macromorphological features, basidiomata images were taken using a Canon EOS 6D Mark II camera (Canon, Tokyo, Japan) fitted with a 100 mm macro lens, and the habitat, altitude, substrate, and nearby vegetation were documented. Basidiomata were dried in a hot air dehydrator (45 C) or in allochroic silica gel for their preservation. Color notation of fresh basidiomata is according to Kornerup and Wanscher (1978), with color codes noted in parentheses (e.g., 6E8, 7E5–6). Details of the basidioma surface were examined under a Zeiss Stemi 305 stereo microscope (Carl Zeiss, Oberkochen, Germany).

Microscopic analyses were performed from sections of fresh or rehydrated tissues under a Zeiss Axioscope 5 microscope (Carl Zeiss) with bright-field, phase-contrast optics, and a charge-coupled device camera (Zeiss Axiocam 208 color; Carl Zeiss). Dried samples were rehydrated in distilled water or moistened in ethanol 70%, and microsections were cut by hand with a razor blade. Microscopic structures were examined in water, 3% KOH, 1% phloxine B ($C_{20}H_4Br_4Cl_2K_2O_5$), cotton blue, and Melzer's reagent, separately. At the beginning of a set of basidiospore data, the abbreviation [a/b/c] signifies "a" basidiospores measured from "b" basidiomata of "c" collections. Dimensions of basidiospores are presented in the following form: (m–)n–o(–p), in which "m" is the smallest value observed or calculated and "p" is the largest value observed or calculated. In the range of values observed or calculated, the 5th percentile is "n" and the 95th percentile is "o." Biometric variables are defined as follows:

L_m (W_m) = the average of all lengths (widths) of basidiospores for each collection, presented as a range of values.

Q = the ratio of length to width of a basidiospore or the range of such ratios for all basidiospores measured.

Q_m = the average of all Q values computed for each collection, presented as a range of values.

The form of the basidiospores was interpreted based on the Q values, following Bas (1969), and descriptive terms for other morphological features follow Vellinga and Noordeloos (2001) and Spirin et al. (2023). Morphological characters from previously described *Pseudohydnum* species included in the dichotomous key are according to Zhou et al. (2022).

The width of the basidia was taken at the widest part, and the length was measured from the apex without sterigmata to the basal septum.

The collections examined including the selected nomenclatural types are deposited at the Fungarium IFungiLab (FIFUNGI) from the Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), and some duplicates were deposited at the herbarium SP. The herbaria abbreviations follow Thiers (2024, continuously updated).

DNA extraction, PCR amplification, and sequencing.—Genomic DNA was extracted from basidiomata dried in silica gel or from herbarium specimens, according to the instructions of the DNeasy Plant Mini Kit (Qiagen, San Diego, California) or the GenElute Plant Genomic DNA Miniprep Kit (MilliporeSigma, Burlington, Massachusetts).

The polymerase chain reactions (PCRs) were conducted on a Mastercycler Nexus GX2 thermal cycler (Eppendorf, Hamburg, Germany). The following primers were used for amplification and sequencing: ITS1F/ITS4, ITS1/ITS4B, or ITS8F/ITS6R (Dentinger et al. 2010; Gardes and Bruns 1993; White et al. 1990) for the nuc rDNA internal transcribed spacer region (ITS: ITS1-5.8S-ITS2); LR0R/LR5 or LR0R/LR7 (James et al. 2006; Vilgalys and Hester 1990) for part of the nuclear ribosomal large subunit (also known as nuc 28S rDNA gene, 28S or LSU); and gRPB1-Af/fRPB1-Cr (Matheny et al. 2002; Stiller and Hall 1997) for part of the RNA polymerase II largest subunit (*RPB1*). The amplification reaction mixture contained 25 µL Taq Pol Green Master Mix (2×) (Cellco Biotech, São Carlos, Brazil), 2.5 µL of each primer, 15 µL of ultrapure water, and 5 µL of template DNA. The thermal profile of PCR for rDNA markers was according to Binder and Hibbett (2003). The amplification of the protein-coding gene *RPB1* employed a touchdown PCR protocol, as detailed by Justo and Hibbett (2011). After visualization of positive PCR products on a 2% agarose gel with SafeDye Nucleic Acid Stain (Cellco Biotech, São Carlos, Brazil), PCR products were cleaned up prior to sequencing using QIAquick PCR Purification Kit (Qiagen) or PCR Purification Kit DPK-106 (Cellco Biotech, São Carlos, Brazil) as per manufacturer's guidelines.

Sequencing (Sanger method) was carried out at the Centro de Estudos do Genoma Humano e Células-Tronco at the Universidade de São Paulo (CEGH-USP, São Paulo, Brazil) or at Macrogen Korea (Seoul, South Korea). Sequencing was carried out using the same primers as those used for PCR.

Alignment and molecular phylogenetic analyses.—Bidirectional sequencing results were assembled using Geneious Prime 2022.1.1 (Biomatters, Auckland, New Zealand). Low-quality nucleotide sites at both ends of the sequences were pruned. All new sequences from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/). Sampling for reference sequences of *Pseudohydnum* as well as for the

phylogenetic backbone of *Auriculariales* was according to Lutzoni et al. (2004), Porter et al. (2008), Malysheva and Spirin (2017), Yuan et al. (2018), Liu et al. (2022), Li et al. (2022), Zhou et al. (2022), Zhou et al. (2023), and Spirin et al. (2023), or selected by using the BLASTn search function of the National Center for Biotechnology Information (NCBI) database to find similar matches with taxa in *Pseudohydnum*. The supplementary table gives all taxa, collection information, and GenBank numbers of sequences used in this study (SUPPLEMENT 1).

Three data sets were assembled for this study. Data set I (ITS+28S), containing representatives of all *Pseudohydnum* species with molecular sequences available as well as the backbone species of Auriculariales, was used to evaluate the phylogenetic placement of *Pseudohydnum* species in an order-level scheme. This data set was concatenated and included sequences of *Bjerkandera adusta* (Willd.) P. Karst. and *Sistotrema brinkmannii* (Bres.) J. Erikss. as outgroup (Li et al. 2022). Data set II (ITS+28S) encompasses virtually all available sequences of *Pseudohydnum* species (very short and/or incongruent sequences were excluded) and sequences of *Protomerulius substuppeus* (Berk. & Cooke) Ryvarden and *Protomerulius subreflexus* (Lloyd) O. Miettinen & Ryvarden as outgroup. This second data set was employed to gather only *Pseudohydnum* sequences for a more optimized alignment by preventing the loss of information due to the high variability in the ITS region and misaligned blocks in ITS matrix when sequences of highly diverse species are included in a single data set (Oliveira et al. 2020). Data set III (ITS +28S+*RPB1*) comprises all *Pseudohydnum* representatives with *RPB1* sequences available in GenBank, thereby representing the vast majority of the genus' lineages in an outgroup-free (midpoint rooting) scheme.

Alignments for individual gene regions were made using MAFFT 7490 as implemented in Geneious (Katoh et al. 2002; Katoh and Standley 2013), adjusting the direction of nucleotide sequences according to the first sequence and selecting the E-INS-i iterative refinement method. Alignments were manually adjusted with AliView 1.17.1 (Larsson 2014) to minimize ambiguously aligned sites. Individual marker alignments were concatenated using Mesquite 3.70 (Maddison and Maddison 2023). The conflicts between the three genes under consideration were tested using PAUP* 4.0b10 (Swofford 2002). The results of the phylogenetic signals in the three genes were not in conflict. The concatenated data set comprising two loci was divided into four data partitions: ITS1, 5.8S, ITS2, and 28S, whereas the data set comprising three loci was divided into three partitions: ITS, 28S, and *RPB1*. The combined alignments produced in this study can be found in TreeBASE (http://www.treebase.org/treebase-web/home.html; submission ID 31094) and as NEXUS file in Supplementary Material (SUPPLEMENT 2).

The phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian inference (BI) in the CIPRES Scientific Gateway (https://www.phylo.org/portal2/home.action) (Miller et al. 2010). Maximum likelihood bootstrap analysis for phylogeny and assessment of branch support by bootstrap percentages (% BS) was carried out using RAxML-HPC 8 on XSEDE (8.2.12) (Stamatakis 2014) under the GTR+GAMMA model with 1000 bootstrap iterations. Bayesian analysis for the reporting of Bayesian posterior probability (BPP) support for branches was performed using MrBayes 3.2.7 on XSEDE (3.2.7a) (Ronquist et al. 2012). The best-fit nucleotide substitution model for each marker partition was determined with ModelTest 2 2.1.6, based on the Bayesian information criterion (BIC) (Posada and Crandall 1998). Four simultaneous Markov chains were run for 10 000 000 generations, and trees were sampled every 1000th generation (resulting in 10 000 trees). The first 25% trees, which represented the burn-in phase of the analysis, were discarded. The BPPs in the majority rule consensus tree were calculated by the remaining trees.

Phylogenetic trees from the ML and BI analyses were visualized with FigTree 1.4.4 (Rambaut 2018) and edited in CorelDRAW 2023 (Alludo, Ottawa, Canada) and Adobe Photoshop CC 2023 (Adobe Systems, São José, California). Branches that received BS and BPPs greater than or equal to 70% and 0.95, respectively, were considered significantly supported.

RESULTS

Phylogenetic relationships.—For data set I, the final alignment consisted of 196 sequences, comprising 37 newly generated sequences (18 ITS and 19 28S) and 159 sequences (83 ITS and 76 28S) downloaded from GenBank. The final alignment contained 1888 nucleotide sites including gaps (311 sites for ITS1, 176 sites for 5.8S, 319 sites for ITS2, and 1079 sites for 28S), of which 920 were conserved, 703 were parsimony-informative, and 265 were variable but parsimony-uninformative. For BI, the selected models for each DNA region of the concatenated data set were as follows: TPM2uf+G for ITS1 and ITS2, TPM2+I+G for 5.8S, and TIM1ef+I +G for 28S. The BI and ML analyses resulted in similar topologies, except the clade formed by *P. umbrosum*,

which changed to be basal to the /*gelatinosum* clade in the ML tree instead of forming a single-species lineage in a polytomy at the /*gelatinosum* clade root in the BI tree. However, basal status of *P. umbrosum* in ML analysis does not get statistical support; thus, only the BI topology is presented as a master tree (FIG. 2) with BPP (≥0.95, first) and BS (≥70%, second) values labeled along the branches. In the boundaries delineated (FIG. 2), the genus *Pseudohydnum* forms a well-supported lineage (BPP = 0.95, BS = 92%) in Auriculariales, which splits into two major and well-supported clades referred to here as clade /*gelatinosum* (BPP = 1, BS = 93%) and clade /*viridimontanum* (BPP = 0.99, BS = 100%). The former encompasses 16 previously known species as well as three species new to science, formally introduced below (see Taxonomy) as *P. brasiliense, P. brunneovelutinum*, and *P. cupulisnymphae*. Meanwhile, the latter clade is a single-species lineage represented by a new taxon described herein as *P. viridimontanum*. This unexpected placement of the /*viridimontanum* clade at first gave rise to a proposal to split *Pseudohydnum* into two subgenera or even consider the *P. viridimontanum* lineage to be a putative distinct genus, but this phylogenetic placement was not corroborated based on the hypothesis inferred from data sets II and III. Furthermore, a thorough morphological analysis of *P. viridimontanum* collections has failed to identify any macro- and microanatomical differences that could justify any segregation from *Pseudohydnum*.

Despite some differences in branch relationships, analyses of the data set II, including virtually all available sequences of *Pseudohydnum*, resulted in the same lineages resolved in the topologies from data set I. The final alignment contained 2058 nucleotide sites including gaps (262 sites for ITS1, 170 sites for 5.8S, 277 sites for ITS2, and 1349 sites for 28S), of which 1545 were conserved, 391 were parsimony-informative, and 122 were variable but parsimony-uninformative. Substitution models were as follows: GTR+G (ITS1), TrNef (5.8S), TPM3uf+G (ITS2), and TIM1+I+G (28S). Phylogenetic trees obtained from ML and BI analyses were similar in overall topologies and were not significantly different. The BI tree is shown in FIG. 3. Sixteen previous *Pseudohydnum* species plus the four new taxa proposed here and some unidentified or misidentified collections were recovered in a fully supported clade (BPP = 1, BS = 100%). Eight main lineages (subclades) with high support could be recognized in the ingroup. Of them, seven overlap with those recovered in the recent ITS+28S–based phylogeny of Spirin et al. (2023) and one constitutes a new lineage represented by *P. brasiliense, P. brunneovelutinum*, and *P. cupulisnymphae. Pseudohydnum viridimontanum* is nested within lineage 1 (BPP = 0.99), alongside *P. umbrosum* from East Asia. This phylogenetic placement was not congruent with the topology retrieved from data set I, which resolves *P. umbrosum* as single-species lineage in the /*gelatinosum* clade, whereas *P. viridimontanum* lay in a distinct lineage (/*viridimontanum*) basal to the *Pseudohydnum* core clade.

Among all the resolved lineages, the second and third (FIG. 3) demand a more careful taxonomic interpretation, which is corroborated by Spirin et al. (2023) in a study that regarded both lineages as species complexes. In our analyses, lineage 2 is represented by *P. translucens* (= *P. candidissimum*) from East Asia, *P. himalayanum* and *P. sinobisporum* from China, *P. alienum* from Europe, and *P. omnipavum* from the northwestern part of North America. In addition, two sequences identified as "*P. gelatinosum*" from Canada (MN992495 and MN992496) and two highly similar sequences of *Pseudohydnum* sp. from Mexico (KC152166 and KT875091) were recovered in this lineage. As reconstructed (FIG. 3), three main subclades can be distinguished within lineage 2. The first encompasses *P. sinobisporum*, which formed a significantly supported clade (BS = 77%) in the ML tree, appearing as sister to the Mexican *Pseudohydnum* sp. sequences, with strong support (BPP = 0.99, BS = 97%). The second consists of a cluster of sequences belonging to species from the temperate-boreal forests of the Northern Hemisphere, viz., *P. himalayanum*, "*P. gelatinosum*," *P. translucens*, and *P. alienum*. These species nest in a moderately supported clade with *P. alienum* assuming a basal position to the "*P. himalayanum* + "*P. gelatinosum*" + *P. translucens*" branch; all independent clades were well supported (BPP = 1, BS = 100%; BPP = 1, BS = 100%; BS = 76%; BPP = 0.99, BS = 76%; respectively). Finally, in the third subclade, *P. omnipavum* appears as a single-species lineage with full support (BPP = 1, BS = 100%). In lineage 2, "*P. gelatinosum*" (QFB28581, QFB28623) from Canada and *Pseudohydnum* sp. (CB 08107, GO-2009-433) from Mexico clearly represent undescribed species and, thus, require proper morphological studies to support their recognition.

In lineage 3, Eurasian sequences of *P. gelatinosum* s.s. grouped within a well-defined but not strongly supported subclade, sister to the North American *P. gelatinosum* ssp. *pusillum*. The latter is grouped within a well-supported subclade (BS = 87%) in the ML tree, which also includes a number of sequences generically identified as "*P. gelatinosum*" from North America. This phylogenetic placement is consistent

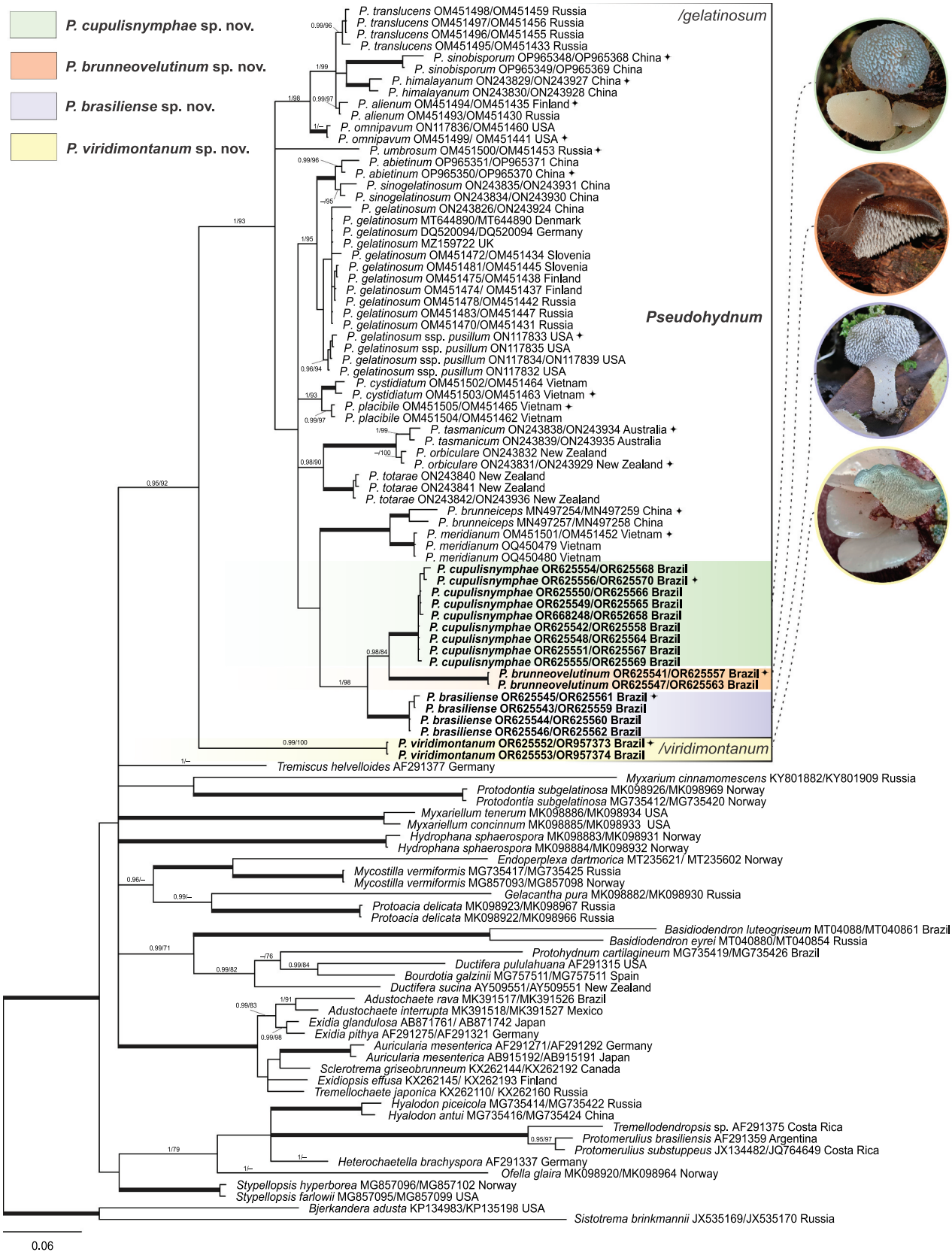

Figure 2. Bayesian inference (BI) tree of *Pseudohydnum* in Auriculariales inferred from the concatenated data set of ITS and 28S regions. GenBank accession numbers and country of origin follow taxon name. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BPP and 1.0 BS support. The tree is rooted to *Bjerkandera adusta* (HHB-12826-Sp) and *Sistotrema brinkmannii* (isolate 236). The new species are presented in bold under color-highlighted clades, with an indicative legend in the upper left and illustration of representative specimen in the right. Black stars represent holotype specimens.

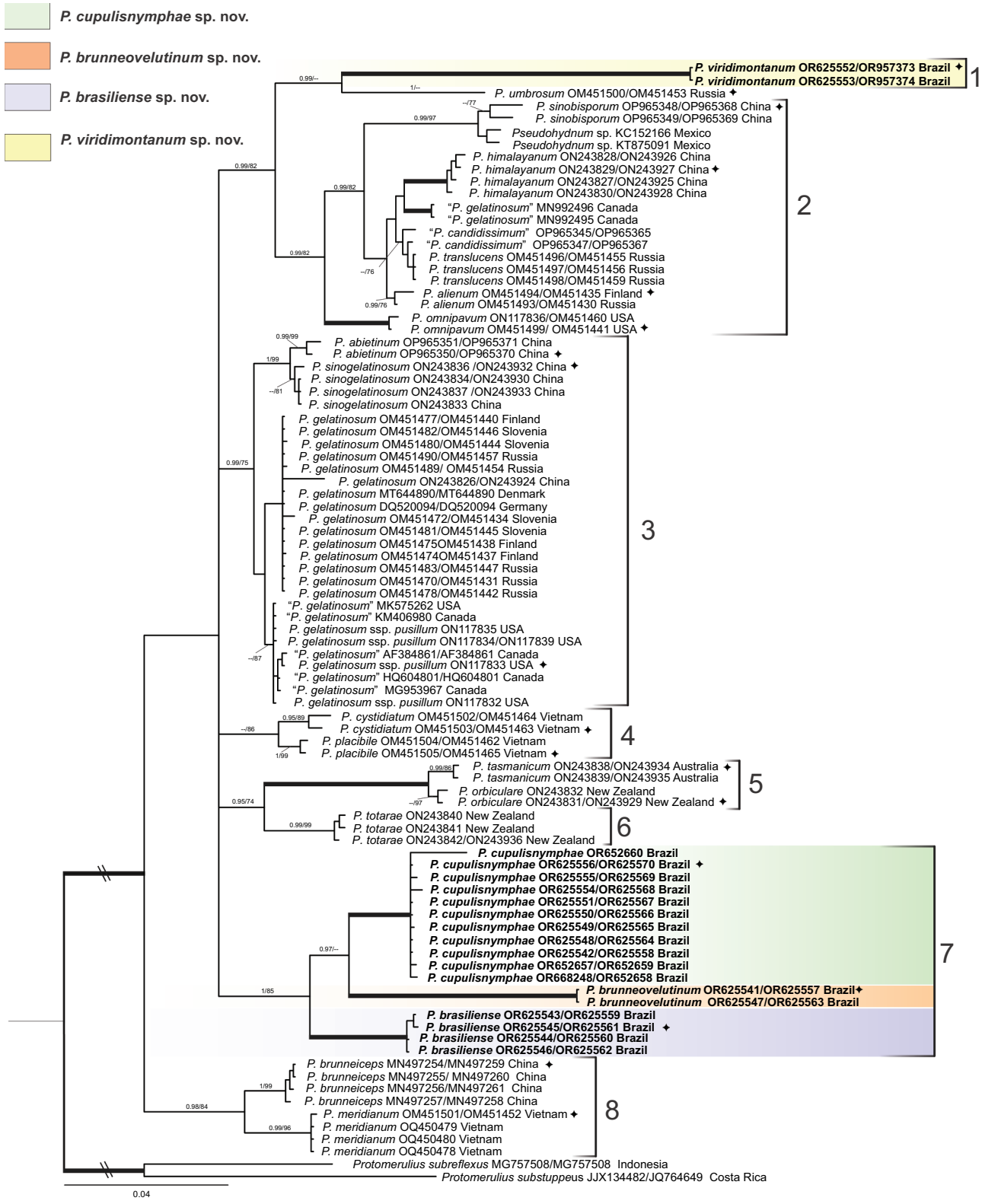

Figure 3. Combined phylogenetic ITS+28S topology from Bayesian analysis for *Pseudohydnum* spp. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BS and 1.00 BPP support. The eight main lineages inferred are enumerated. The tree is rooted to *Protomerulius subreflexus* (OM 14402.1) and *P. substuppeus* (O 19171). The new species are presented in bold under color-highlighted clades, with an indicative legend in the upper left and illustration of representative specimen in the right. Black stars represent holotype specimens.

with that of the data set I tree, in which the *P. gelatinosum* ssp. *pusillum* clade was strongly supported (BPP = 0.96, BS = 94%). *Pseudohydnum abietinum* and *P. sinogelatinosum* also cluster in the third lineage, appearing as sisters to the branch bearing "*P. gelatinosum* s.s. + *P. gelatinosum* ssp. *pusillum*," with significant support (BPP = 0.99, BS = 75%).

Finally, for data set III (ITS+28S+*RPB1*), the final alignment consisted of 87 sequences, including six newly generated *RPB1* sequences. The final alignment contained 2631 nucleotide sites (604 sites for ITS, 894 sites for 28S, and 1133 for *RPB1*), of which 2160 were conserved, 415 were parsimony-informative, and 56 were variable but parsimony-uninformative. Substitution models were as follows: TIM2ef+G (ITS1), GTR+I+G (LSU), and TrN+I (*RPB1*). The overall topologies generated through ML and BI analyses were found to be highly consistent, with no significant differences. The BI tree is shown in FIG. 4. The phylogenetic inference from this data set fully supports all the new introduced species, and it is in accordance with the topology derived from data set II.

TAXONOMY

Based on our morphological, ecological, and molecular phylogenetic data, four new species of *Pseudohydnum* from Brazil are described and illustrated here.

Pseudohydnum brasiliense C. Coelho-Nascimento, D. A. Zabin & Menolli, sp. nov.
MycoBank MB851356

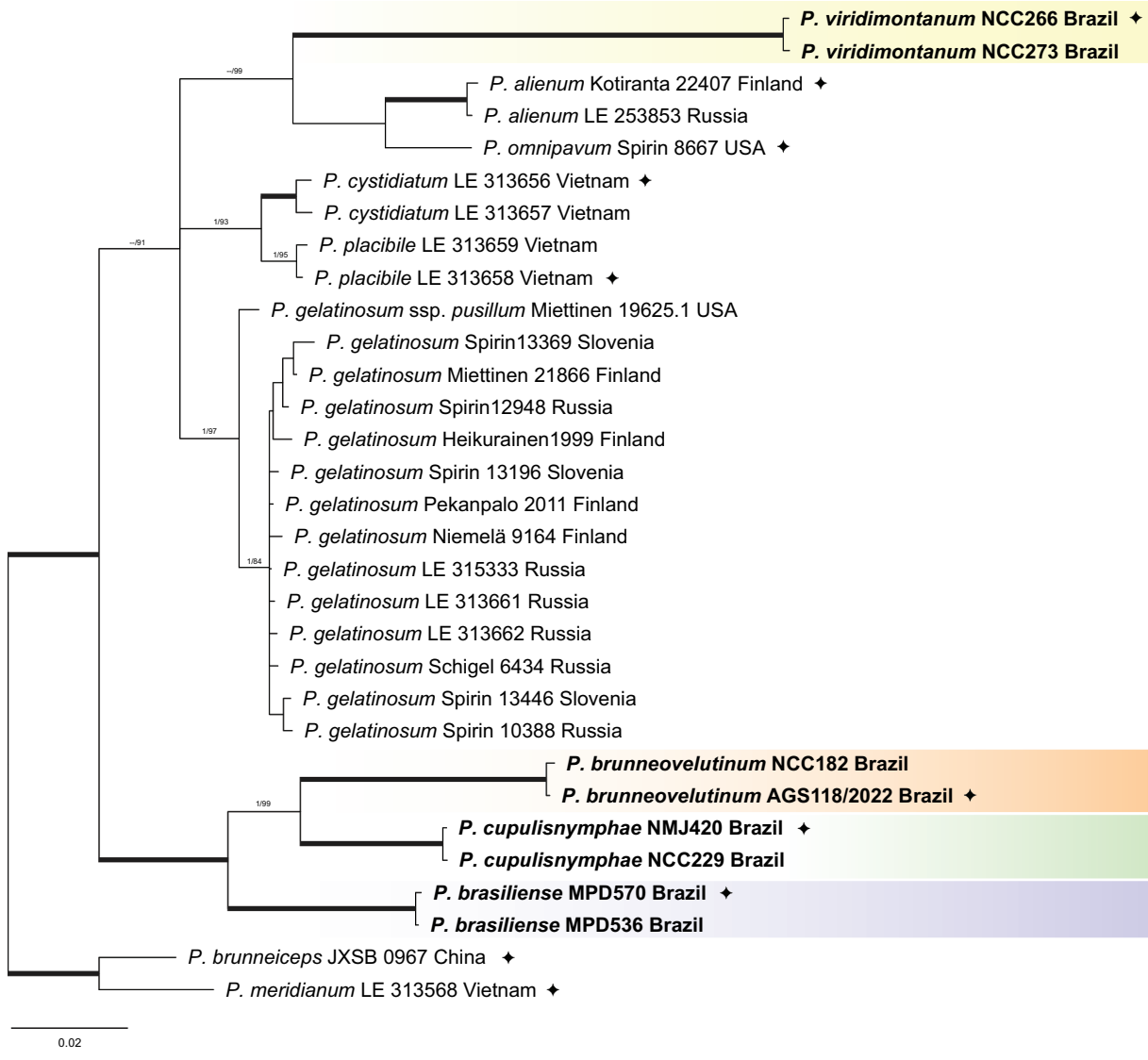

Figure 4. Combined phylogenetic ITS+28S+*RPB1* topology from Bayesian analysis for *Pseudohydnum* spp. The values at each node indicate the BPP (≥0.95) and the BS (≥70%). Thick lines on branches indicate 100% BS and 1.00 BPP support. The tree is midpoint-rooted. The new species are presented in bold under color-highlighted clades. Black stars represent holotype specimens.

Typification: BRAZIL. SÃO PAULO: Campos do Jordão, Parque Estadual Campos do Jordão, Trilha do Rio Sapucaí, from decaying angiosperm wood, 21 Jan 2020, *M.P. Drewinski, N. Menolli Jr., M.F.O. Silva, M.P.C. Santos & T.R. Santos MPD570* (**holotype** FIFUNGI279), GenBank: ITS = OR625545, 28S = OR625561, *RPB1* = PP805851.

Diagnosis: Differs from other known *Pseudohydnum* species in having basidiomata with a lateral well-developed stipe, yellowish gray to grayish brown or fawn brown pileus surface, hymenophoral spines 2.5–4 per mm, simple hyphidia, and subglobose to broadly ellipsoid basidiospores.

Etymology: brasiliense (Latin) refers to the country of the type locality.

Morphology: Basidiomata small- to medium-sized, gelatinous when fresh, pileate, stipitate, solitary or gregarious (not confluent at the base), irregularly subglobose in young stages, becoming more or less trumpet-shaped in profile before the complete pileus expansion. Pileus up to 42 mm, spathulate to flabellate or pseudoinfundibuliform, surface minutely velutinous to pubescent, yellowish gray (4B2) to grayish brown (6D3, 6EC) or fawn brown (7E4), translucent when wet; margin sharp, sometimes undulating. Hymenophore white, hydnoid. Hymenophore spines sharp-tipped, 0.4–2.5 mm long, 2.5–4 per mm, white (1A1) to yellowish white (1A2). Stipe 6–11 × 3.5–6 mm, white (1A1) to yellowish white (1A2) or yellowish gray (4B2), translucent; surface covered by short spines to minutely grandinioid or almost smooth. Context translucent, unchanging. Odor mild. Taste mild or pleasant.

Hyphal system monomitic, generative hyphae with abundant clamp connections, hyphae unchanged in KOH. Pileal surface trichodermial to subtrichodermial, fascicles abundant of sinuate to slightly sinuate hyphae (3.0–10.0 µm diam) that are colorless or pale grayish brown in mass and frequently septate, thin- to slightly thick-walled; terminal elements poorly differentiated. Contextual hyphae of two types: (i) dominant thin- to slightly thick-walled, smooth, hyaline, interwoven, commonly branching, frequently anastomosing, 1.0–3.0 µm diam; and (ii) occasional thin-walled, slightly inflated up to 15.0 µm diam. Spine tramal hyphae thin-walled, smooth, hyaline, moderately branched, interwoven, 1.5–3.0 µm diam. Hyphidia covering basidial cells, thin-walled, smooth, sometimes slightly flexuous, simple, slightly swollen toward the apex; apex cylindrical to subfusiform, usually slightly constricted, 1.6–2.5 µm diam; hyphidia and basidia derived from the same hyphae. Basidia mostly 2-celled (bisporic), rarely 4-celled, longitudinally septate, multigutullate, barrel-shaped, ellipsoid to subglobose, 9.0–11.3 × 7.0–8.6 µm; sterigmata 4.5–15.2 × 1.6–3.5 µm, swollen or sometimes aciculate, occasionally constricted. Probasidia mostly subglobose to globose or lageniform, 8.0–8.7 × 5.9–7.0 µm, usually multigutullate. Basidiospores [240/8/8] (5.7–)6.4–9.7(–10.5) × (4.7–)5.4–8.8(–9.5) µm [L_m = 7.3–7.9 µm; W_m = 6.2–6.8 µm; Q = (1.02–)1.06–1.32(–1.39); Q_m = 1.16–1.18], subglobose to broadly ellipsoid, rarely globose or ellipsoid, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, often germinating by repetition or by germ tubes on the surface.

Habitat and distribution: Growing erect and ascendant on decayed angiosperm wood in dense and mixed ombrophilous forest fragments of Southeastern Brazil. Also found on *Pinus taeda* L. wood in a seasonal semideciduous forest of Southern Brazil. Known for the Brazilian states of São Paulo, Rio de Janeiro, Rondônia, and Santa Catarina.

Other specimens examined: BRAZIL. RIO DE JANEIRO: Teresópolis, Parque Nacional da Serra dos Órgãos, Trilha da Pedra do Sino, from decaying angiosperm wood, 14 Dec 2019, *M.P. Drewinski, J.A.D. Barbosa, T.S. Cabral & C.S. Carvalho MPD536* (FIFUNGI281), GenBank: ITS = OR625543, 28S = OR625559, *RPB1* = PP805850; ibid., 16 Dec 2019, *M.P. Drewinski, J.A.D. Barbosa, T.S. Cabral & C.S. Carvalho MPD562* (FIFUNGI282), GenBank: ITS = OR625544, 28S = OR625560; SÃO PAULO: Campos do Jordão, Parque Estadual Campos do Jordão, 27 Jan 1987, *D.N. Pegler, K. Hjortstam & L. Ryvarden KH 3858* (SP214396); ibid., 28 Jan 1987, *D.N. Pegler, K. Hjortstam & L. Ryvarden KH 3859* (SP214385); Trilha do Rio Sapucaí, from decaying *Pinus taeda* wood, 21 Jan 2020, *M.P. Drewinski, N. Menolli Jr., M. F.O. Silva, M.P.C. Santos & T.R. Santos MPD572* (FIFUNGI280), GenBank: ITS = OR625546, 28S = OR625562; Cananeia, Ilha do Cardoso, 3 Feb 1987, *M. Capelari, R. Maziero & M.I.S.M.C. Castro 1241* (SP211805); RONDÔNIA: Jarú, Reserva Biológica do Jarú, on the right bank of the Ji-Paraná River, from decaying angiosperm wood, 13 May 1987, *M. Capelari, R. Maziero & M.R.O. Santos MC1456* (SP212177); SANTA CATARINA: Urubici, Reserva Particular do Patrimônio Natural (RPPN) Portal das Nascentes, Trilha da Água, from decaying angiosperm wood, 9 Jan 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, E.L. Gumboski, E.S. Alves, & P.R. Pezzuto MIND.Funga0397* (FLOR 71400), GenBank: ITS = PP790410.

Notes: The presence of a well-developed stipe differentiates *P. brasiliense* from other known species of *Pseudohydnum* and those described in the present paper. It also has a distinct pileus color variation, ranging from yellowish gray (collections MPD570 and MPD572) to fawn brown (collection MPD536), with occasional pale brown to watery-gray tinges in rain washed basidiomata (collections MPD562 and MIND. Funga0397). The fawn brown pileus of *P. brasiliense* in the collections MPD536 and MIND.Funga0397 evoke *P. brunneovelutinum* in the field. However, the latter can be distinguished by a number of characters, including the short to rudimentary stipe, presence of dendrohyphidia, and smaller basidiospores (5.9–6.8 × 5.2–6.3 µm). *Pseudohydnum brasiliense* can also be compared with *P. brunneiceps* because of their similar large basidiospores (6–8.9 × 5.5–7.1 µm, Q_m = 1.11–1.17 in *P. brunneiceps*) and well-developed stipe, but the latter has larger basidiomata with a maximum reported pileus diameter of 80 mm, the hyphidia are richly branched, and its ecology and distribution are different because it is so far known only from the southern and southeastern subtropical zone of China, where it inhabits decayed wood of conifers, mainly *Cryptomeria japonica* (Thunb. ex L. f.) D. Don (Chen et al. 2020; Spirin et al. 2023).

Pseudohydnum brasiliense also deviates from its closest relatives, except *P. brunneiceps*, in the presence of mostly bisporic basidia, unique among the species introduced herein. A very similar collection with remarkable bisporic basidia was described from French Guyana as *P. gelatinosum* var. *bisporum* (Courtecuisse and Lowy 1990). From an anatomical perspective, the latter closely resembles *P. brasiliense* because, in addition to bisporic basidia, both share similar pileal surface hyphae, width of contextual/tramal hyphae, and basidiospores. Conversely, neither macromorphological descriptions nor basidioma line drawings were provided in Courtecuisse and Lowy's protolog. These authors ascribed the same macromorphological concept of the type variety presented by Lowy (1971) for its new varietal name, which includes sessile to short-stipitate basidiomata. This latter trait deviates from the well-developed stipe delivered by *P. brasiliense*. Thus, despite the somewhat divergent macromorphology, we interpret that *P. gelatinosum* var. *bisporum* is likely conspecific with *P. brasiliense*, and the diagnostic well-developed stipe was an overlooked trait in the former's protolog. However, we cautiously refrain from proposing a synonymy between them until the authentic material is fully reexamined to support our viewpoint.

Bastos et al. (2015) identified a collection from the Brazilian Amazon Forest as *P. gelatinosum* based on the description provided by Lowy (1971). However, judging from descriptions and pictures, the specimen from the Amazon Forest barely resembles Lowy's material, from which it differs by the shorter basidiospores (6–7 × 8–5 µm) and the presence of a well-developed stipe (Bastos et al. 2015). With the exception of larger basidiospores, the collections of *P. brasiliense* herein examined mostly agree to that studied by Bastos et al. (2015), especially by having similar pileus dimensions and a well-developed stipe; thus, they may be conspecific, although a careful reexamination of the samples studied by them is necessary in order to confirm this. A second Amazon collection, designated as "*P. gelatinosum*" and revised from herbarium SP (SP212177), is consistent with the *P. brasiliense* morphoanatomical concept, exhibiting a distinctly stipitate basidiomata, bisporic basidia, and subglobose to broadly ellipsoid basidiospores (6.3–8.3 × 5.3–7.2 µm, Q_m = 1.16). Unfortunately, sequences from this old collection could not be obtained to support the morphoanatomical evidence.

In the phylograms depicted (FIGS. 2 and 3), *P. brasiliense* is sister to the "*P. brunneovelutinum* + *P. cupulisnymphae*" clade, with which all together forming a well-supported lineage (BPP = 1, BS = 85%) of Neotropical species. Nevertheless, no shared macro- and microanatomical traits were identified between these three species that could support their clustered phylogenetic placement, except the basidiospores commonly germinating by repetition. However, this latter feature should not be overemphasized in taxonomic terms, since it seems to be a common trait present in a number of *Pseudohydnum* species. Differences of *P. brasiliense* from the phylogenetically close *P. cupulisnymphae* are discussed under this species.

Pseudohydnum brunneovelutinum C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 8, 9
MycoBank MB851357

Typification: BRAZIL. ESPÍRITO SANTO: Santa Teresa, Reserva Biológica Augusto Ruschi, from decaying angiosperm wood, 19 Nov 2022, *A.G.S Silva-Filho & C.A.V. Fraga Jr. AGS118/2022* (**holotype** FIFUNGI283), GenBank: ITS = OR625541, 28S = OR625557, *RPB1* = PP805852.

Diagnosis: Differs from other known *Pseudohydnum* species in having flabelliform to petaloid basidiomata with a short to rudimentary stipe base, cocoa brown to brown or dark brown pileus surface, sparse spines up to

1.5 per mm at base, conspicuous dendrohyphidia, and globose to broadly ellipsoid basidiospores.

Etymology: brunneo (Latin) = brown; *velutinum* (Latin) = velvety, densely covered with fine, short, soft, erect hairs; referring to the brown velutinous pileal surface of the specimens.

Morphology: Basidiomata small-sized, gelatinous when fresh, growing solitary or in pairs (then basally coalescent), at first as a protruding jelly tongue with an incurved edge, then flabelliform to petaloid with a short stipe. Pileus up to 35 mm in widest dimension, surface initially smooth, then velutinous to pubescent, cocoa brown (6E6) to brown (6E8, 7E5–6) or dark brown (5F6–8, 6F6–8), not translucent; margin sharp, even, fertile. Hymenophore white, hydnoid. Hymenophore spines sharp-tipped, white (1A1), up to 1.5 per mm, of two types: (i) longer well-developed spines, 1–2 mm long; and (ii) very small spines up to 0.3 mm long. Stipe short, up to 15 mm long, sometimes rudimentary, concolor with pileal surface; surface covered by spines or minutely papillate. Context translucent, pale brownish gray (9C2) to pale grayish brown (9D3), unchanging. Odor and taste mild.

Hyphal system monomitic, generative hyphae with abundant clamp connections, hyphae unchanged in KOH. Pileal surface as a compact trichoderm, with abundant fascicles of interwoven and vertically projected hyphae; hyphae 2.5–5 µm diam, thin- to slightly thick-walled, sometimes sinuous to tortuous, sparingly branched. Contextual hyphae thin- to slightly thick-walled, hyaline, colorless to pale yellow, frequently branching, straight to tortuous, interwoven, 1.8–3.5 diam. Spine tramal hyphae thin-walled, commonly branching, interwoven, 1.5–2.8 µm diam. Hyphidia conspicuous, strongly and irregularly branched (dendrohyphidial), thin-walled, slightly thick-walled toward the base, smooth, hyaline, partially covering basidia but sometimes projecting above hymenial layer up to 30 µm. Basidia mostly 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose or globose, 10.1–12.8 × 7.2–10.4 µm; sterigmata up to 20.6 × 2.2 µm, conic to aciculate or distinctly longer and tubiform with an obtuse apex. Probasidia subglobose to pyriform or short clavate, 9.0–10.5 × 4.7–8.4 µm, mostly multigutullate. Basidiospores [120/4/2] (5.3–)5.9–6.8(–7.7) × (4.6–)5.2–6.3(–6.7) µm [L_m = 6.3–6.5 µm; W_m = 5.7–5.8 µm; Q = (1.00–)1.02–1.38 (–1.51); $\mathbf{Q_m}$ = 1.11–1.15], globose to broadly ellipsoid, infrequently ellipsoid, inamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, occasionally germinating by repetition.

Habitat and distribution: Growing on unknown decayed angiosperm wood in dense ombrophilous forest fragments of Southeastern Brazil. Known for the Brazilian states of Espírito Santo and São Paulo.

Other specimen examined: BRAZIL. SÃO PAULO: São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, Trilha das Figueira, from decaying angiosperm wood, 4 Feb 2022, *C. Coelho-Nascimento NCC182* (FIFUNGI284), GenBank: ITS = OR625547, 28S = OR625563, *RPB1* = PP805853.

Notes: Pseudohydnum brunneovelutinum deviates from all other known *Pseudohydnum* species due to the presence of conspicuous dendrohyphidia, which is unique within the genus. Thus, it can hardly be mistaken by microscopic examination, even in the absence of molecular information.

From a macromorphological point of view, *P. brunneovelutinum* may evoke *P. orbiculare* in the field because of the dark brown and velutinous pileus surface and the hymenophore with sparse spines, but the latter, described from New Zealand's South Island, has sessile basidiomata and larger basidiospores, 6.5–7.9 × 5.6–6.8 µm (Zhou et al. 2022). Because of their similar pileus surface color, *P. brunneiceps, P. meridianum*, and *P. umbrosum* can also be compared with *P. brunneovelutinum*. However, *P. brunneiceps* and *P. umbrosum*, both species poorly sampled from the Asian continent (Chen et al. 2020; Spirin et al. 2023), differ from *P. brunneovelutinum* by the denser hymenophoral spines and much larger basidiospores, 7.6–9.8 × 6–7.1 µm for *P. brunneiceps* and 6.9–8.9 × 6.1–7.1 µm for *P. umbrosum* (Chen et al. 2020; Spirin et al. 2023), whereas *P. meridianum*, presently known from Vietnam, can be distinguished from *P. brunneovelutinum* by a stouter habit, the pronounced vertical stipe, and the nearly smooth pileal surface, as well as slightly smaller basidiospores (5.1–6.2 × 4.9–5.8 µm) with lower Q values (Q = 1.0–1.1) (Spirin et al. 2023).

Phylogenetically, *P. brunneovelutinum* was recovered as sister to *P. cupulisnymphae* with high support (BPP = 0.98, BS = 84%). Differences between these phylogenetically close species are discussed under *P. cupulisnymphae*.

Pseudohydnum cupulisnymphae C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 10, 11
MycoBank MB851358

Typification: BRAZIL. RIO DE JANEIRO: Paraty, trail to Sítio São José, Parque Nacional da Serra da Bocaina, from decaying angiosperm wood, 3 Dec 2021,

N. Menolli Jr., N. Rigo, A. Sandoval & J. Ferreira NMJ420 (**holotype** FIFUNGI285), GenBank: ITS = OR625556, 28S = OR625570, *RPB1* = PP805855.

Diagnosis: Differs from other known *Pseudohydnum* species in having mostly sessile basidiomata, orbicular to conchiform or irregularly lobed pileus, whitish to grayish yellow or pale pinkish orange pileus surface, hymenophoral spines 3.5–6 per mm, simple to sparingly branched hyphidia, and subglobose to broadly ellipsoid basidiospores (5.8–8.0 × 5.1–7.0 µm).

Etymology: cupulis (Latin) = cup-like shape; *nymphae* (Latin) = fairy; referring to the distinct cup shape of young basidiomata that resembles mystic vessels offered by fairy folk in "Fairy cup legends" (Migratory Legends of Christiansen Type 6045).

Morphology: Basidiomata small to somewhat large-sized, gelatinous when fresh, pileate, sessile to substipitate, solitary or in grouped tiers, sometimes basally coalescent and sharing an extended attachment base, at first forming a globose primordium with a short peduncle, then distinctly funnel-shaped or cupulate, and finally orbicular, flabelliform to flat conical or irregularly shaped. Pileus 13–40(–90) mm diam, surface initially covered by crowded tiny spines, becoming smooth to minutely velutinous or papillate, whitish to grayish yellow (1B3, 3B2) or pale pinkish orange (5A2, 6A3), translucent; margin blunt, even to crenate or irregularly lobed. Hymenophore white to yellowish white, hydnoid. Hymenophore spines sharp-tipped, 1–3 mm long, 3.5–6 per mm, white. Stipe absent or rudimentary. Context up to 15 mm thick, translucent, unchanging. Odor mild or sweet at first, then pleasantly fruity like apricots. Taste mild.

Hyphal system monomitic, composed of clamped generative hyphae, sometimes distinct clamps only in the hymenium, hyphae unchanged in KOH. Pileal surface subtrichodermial to trichodermial, composed of abundant fascicles of cylindrical hyphae (2.5–8.5 µm diam) that are colorless or pale yellowish in mass and frequently septate, thin- to slightly thick-walled; terminal elements narrowly clavate to conical or narrowly utriform. Contextual hyphae thin- to slightly thick-walled, hyaline, branching, interwoven, frequently anastomosing, some with incrustations, 2.0–3.6 µm diam. Spine tramal hyphae thin-walled, smooth, hyaline, commonly branched, interwoven, 1.0–4.2 µm diam. Hyphidia thin- to slightly thick-walled, smooth, rather constant in wide throughout their length, 1.0–3.0 µm diam at the apex, hyaline, spread among basidia and basidioles, mostly simple, sometimes sparingly branched, flexuous. Basidia strictly 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose or globose, sometimes broadly clavate, 10.0–14.0 × 8.5–11.0 µm; sterigmata up to 24 × 2.6 µm, tubiform, usually abruptly tapering at the apex. Probasidia subglobose to short clavate, 9.0–11.4 × 6.1–8.4 µm, mostly multigutullate. Basidiospores [420/14/14] (5.2–)5.8–8(–8.5) × (4.8–)5.1–7(–7.4) µm [L_m = 6.3–6.6 µm; W_m = 5.7–5.9 µm; Q = (1.00–)1.02–1.26(–1.28); Q_m = 1.10–1.18], subglobose to broadly ellipsoid, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, occasionally germinating by repetition.

Habitat and distribution: Common on rotten angiosperm wood in dense and mixed ombrophilous forest fragments of Southeastern and Southern Brazil; other recorded substrate includes the caudice of the tree fern *Dicksonia sellowiana* Hook. ("Samambaiaçu"). Known for the Brazilian states of Paraná, São Paulo, Santa Catarina, Rio de Janeiro, and Rio Grande do Sul, with putative records to Argentina, Bolivia, Guyana, Jamaica, Mexico, and Panama (Lowy 1959, 1971; Niveiro and Popoff 2011).

Other specimens examined: BRAZIL. PARANÁ: Morretes, Parque Estadual Pico do Marumbi, Trilha Antoninho Palmiteiro, from decaying angiosperm wood, 18 Jan 2023, *C. Coelho-Nascimento, A.G.S. Silva-Filho & M.D. Xavier NCC246* (FIFUNGI289), GenBank: ITS = OR625549, 28S = OR625565; ibid., 19 Jan 2023, *C. Coelho-Nascimento, A.G.S. Silva-Filho & M. D. Xavier NCC247* (FIFUNGI286), GenBank: ITS = OR625550, 28S = OR625566; RIO DE JANEIRO: Teresópolis, Parque Nacional da Serra dos Órgãos, Trilha Cartão Postal, from caudice of the tree fern *Dicksonia sellowiana*, 9 Dec 2022, *A.G.S. Silva-Filho, A. L.V. Ribeiro, M.E.C. Morais & C. Coelho-Nascimento NCC229* (FIFUNGI290), GenBank: ITS = OR625548, 28S = OR625564, *RPB1* = PP805854; RIO GRANDE DO SUL: Carambá do Sul, Parque Nacional dos Aparados da Serra, Trilha Fortaleza, from decaying angiosperm wood, 27 May 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, G. Alves-Silva & E.L. Gumboski MIND.Funga0893* (FLOR71600), GenBank: ITS = OR652657, 28S = OR652659; SANTA CATARINA: Benedito Novo, Pousada Campo do Zinco, from decaying angiosperm wood, 24 Nov 2020, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, G. Alves-Silva & E.L. Gumboski MIND.Funga0040* (FLOR71289), GenBank: ITS = OR668248, 28S = OR652658; Urubici, RPPN Portal das Nascentes, Trilha da Água, from decaying angiosperm wood, 9 Jan 2021, *T. Kossmann, M. Titton, E.R. Drechsler-Santos, E. Gumboski, E. dos Santos Alves, & P.R. Pezzuto MIND.Funga0348*

(FLOR71400), GenBank: 28S = OR652660; Brusque, from decaying wood, 14 May 1964, *P. Reitz & K. Wells n/a* (SP71410); SÃO PAULO: São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, Trilha da Bica, from decaying angiosperm wood, 26 Feb 2021, *A.L.V. Ribeiro, M.P.C. Santos, A.C. Morais, C. Coelho-Nascimento, J.F. Amorim & A.M. Oliveira NMJ394* (FIFUNGI291), GenBank: ITS = OR625554, 28S = OR625568; Iporanga, Parque Estadual Turístico do Alto Ribeira, from decaying angiosperm wood, 4 Mar 2021, *N. Menolli Jr., M.P.C. Santos, D.A. Zabin, C. Coelho-Nascimento & N. Rigo NMJ398* (FIFUNGI294), GenBank: ITS = OR625555, 28S = OR625569; Cananeia, Parque Estadual da Ilha do Cardoso, Núcleo Perequê, Trilha Poço das Antas, from decaying angiosperm wood, 8 Jan 2019, *M.P. Drewinski MPD344* (FIFUNGI387), GenBank: ITS = OR625542, 28S = OR625558; Trilha da Cachoeira Grande, from decaying angiosperm wood, 1 Feb 2023, *C. Coelho-Nascimento, N. Menolli Jr. & A.G.S Silva-Filho NCC259* (FIFUNGI288), GenBank: ITS = OR625551, 28S = OR625567; Barra do Turvo, from decaying wood, 6 Dec 1973, *D.M. Vital n/a* (SP112455); São Vicente, Praia de Paranapuã, from decaying wood, 27 May 1993, *D. Matheus & L.K. Povineli CCB 432* (SP250989).

Notes: Pseudohydnum cupulisnymphae has been the most frequently encountered *Pseudohydnum* species in the sampled Atlantic Rainforest areas. It occurs from areas of dense ombrophilous forest in the state of Rio de Janeiro to areas of mixed ombrophilous forest in the state of Rio Grande do Sul. The funnel-shaped or cupulate young basidioma stages represent a diagnostic character that can differentiate *P. cupulisnymphae* from other species herein described. The other striking trait is the stout habitus and markedly lobed pileus margin displayed in the holotype collection (NMJ420; FIG. 11), although the basidioma size in the other studied collections did not exceed a maximum diameter of 40 mm.

Morphological segregation from taxa with overlapping geographic range requires comparisons of *P. cupulisnymphae* with *P. brasiliense* and *P. viridimontanum*. The first can be separated from *P. cupulisnymphae* by its stipitate basidiomata, which display a spathulate to pseudoinfundibuliform pileus, and larger basidiospores (6.4–9.7 × 5.4–8.8 µm) from mostly bisporic basidia. Differences from *P. viridimontanum* are discussed under this species.

For species without an overlapping geographic range, we limit the comparisons to only with other sessile *Pseudohydnum* species, such as *P. orbiculare, P. cystidiatum*, and *P. umbrosum*. Despite similar micromorphology, *P. orbiculare* is easily distinguished by its brownish pileus and very sparse hymenial spines (0.5–1 per mm) (Zhou et al. 2022). On the other hand, *P. cystidiatum*, although it shares with *P. cupulisnymphae* similar pileus color, produces distinctly smaller basidiomata (up to 1 cm diam) and larger basidiospores (6.8–9.5 × 5.6–7.6 µm) (Spirin et al. 2023). Lastly, *P. umbrosum* differs by having smaller basidiomata (up to 2 cm diam), a grayish brown pileus, and larger basidiospores (7.6–9.8 × 6–7.1 µm) (Spirin et al. 2023).

In arguments for novelty, we are also concerned with segregation/association of *P. cupulisnymphae* from previous Neotropical collections named as *P. gelatinosum*. Möller (1895) was the first to describe collections of *Pseudohydnum* [as *Tremellodon* (Pers.) Fr.] in Brazil from specimens found in Blumenau (Santa Catarina state). No authentic material was referenced, but illustrations are available (Möller 1895:tafel V, fig. 34). In Möller's description, a grayish, sessile, and stout specimen (up to 10 cm in diam) growing on rotten wood was identified as *Tremellodon gelatinosum* Pers. (= *Pseudohydnum gelatinosum*). He regarded *T. gelatinosum* as a common and widespread species and considered the Brazilian samples identical to the European look-alike. Judging from his description (Möller 1895), Möller's sessile samples named *T. gelatinosum* are clearly conspecific with *P. cupulisnymphae* because the two share similar habit, basidioma color and size, and basidiospore shape. However, *P. gelatinosum* s.s. from Europe exhibits a smaller pileus (not exceeding 40 mm), commonly stipitate basidiomata, and longer basidiospores (5.3–9 µm) (Spirin et al. 2023). Moreover, some collections of *P. gelatinosum* s.s display a grayish-brownish pileus surface (Spirin et al. 2023), whereas brownish tinges were absent in all examined *P. cupulisnymphae* collections.

Descriptions of collections named as *P. gelatinosum* provided by Lowy (1959, 1971), based on collections from Bolivia, Brazil, Guyana, Jamaica, Mexico, and Panama, also immediately evoke *P. cupulisnymphae*, both macromorphologically and microanatomically, although broader basidiospore dimensions were reported for the collection described as *P. gelatinosum* var. *paucidentatum* (7–9 × 6–8.5 µm) when compared with *P. cupulisnymphae* basidiospore measurements. In addition to the atypical broader basidiospores, Lowy (1959) regarded the widely sparse spines as a distinctive trait to

introduce the latter varietal name from a single collection from Bolivia. However, distinct sparse spines represent a common trait in some small-sized *Pseudohydnum* specimens, especially in species that exhibit a wide variation in the mature basidioma size, as observed for *P. cupulisnymphae* (FIG. 10G). Additionally, the size range of basidiospores should not be overemphasized as sole criterion for taxon segregation in *Pseudohydnum*, as it can exhibit a huge variation, as observed for *P. gelatinosum*, the most common representative of the genus in temperate-boreal forests of Eurasia (Spirin et al. 2023). Thus, based on a cautious interpretation of collections named "*P. gelatinosum*" by Lowy (1959, 1971), we formally consider their collections conspecific with *P. cupulisnymphae* and putatively consider *P. gelatinosum* var. *paucidentatum* to be its synonym, although a revision of the varietal collection type could confirm this.

Finally, we also ascribe the name *P. cupulisnymphae* to the collection reported as *P. gelatinosum* from Argentina by Niveiro and Popoff (2011). The phenotype of the Argentine collection is virtually indistinguishable from *P. cupulisnymphae* because both have similar habit, pileus color, contextual hypha diameter, and ecology.

As interpreted here, the known range of *P. cupulisnymphae* is extended considerably, assuming a wide Neotropical distribution, including, in addition to Brazil, putative records from Argentina, Bolivia, Guyana, Jamaica, Mexico, and Panama (Lowy 1959, 1971; Niveiro and Popoff 2011). Nevertheless, a broader taxon sampling of this species is obviously necessary in order to better understand the full range of its morphoanatomical variability and determine its ecological and biogeographic range more precisely.

Pseudohydnum viridimontanum C. Coelho-Nascimento & Menolli, sp. nov. FIGS. 12, 13
MycoBank MB851359

Typification: BRAZIL. MINAS GERAIS: Camanducaia, Serra da Mantiqueira, Monte Verde, from decaying *Vernonanthura discolor* (Spreng.) H. Rob. wood, 29 Dec 2022, *C. Coelho-Nascimento & T. Comenale NCC266* (**holotype** FIFUNGI292), GenBank: ITS = OR625552, 28S = OR957373.

Diagnosis: Pseudohydnum viridimontanum is similar to *P. cupulisnymphae* but can be distinguished by its smaller and mostly short-stipitate basidiomata, smaller basidiospores (5.8–6.9 × 4.4–5.0 µm), presence of cylindrical to fusiform inflated elements in the spine trama, and absence of clamp connections. In addition, it was only found on *Vernonanthura discolor* wood.

Etymology: viridis (Latin) = green, *montanum* (Latin) = hill, mount, mountain; referring to the type locality, i.e., "Monte Verde" in Portuguese, which means Green Hill.

Morphology: Basidiomata small-sized, gelatinous when fresh, pileate, laterally stipitate or pseudostipitate, solitary or in imbricate clusters, at first as a globose to bell-shaped primordia, then expanding to orbicular, flabelliform or reniform. Pileus up to 35 mm diam, surface smooth to minutely rough, white (1A1) at first, then chalk white to grayish white (1B1 to somewhat paler) or grayish yellow (1B3, 3B2); margin sharp, even to crenate, sometimes undulating. Hymenophore white (1A1) to whitish, hydnoid. Hymenophore spines sharp-tipped, up to 0.8 mm long, 2.5–4 per mm, white (1A1) to translucent white. Stipe short to rudimentary. Context concolorous with the pileus, translucent appearance when wet, thick centrally and gradually thinner toward the margin, unchanging. Odor mild. Taste mild or pleasant.

Hyphal system monomitic, generative hyphae clampless, hyphae unchanged in KOH. Pileal surface subtrichodermial to trichodermial, composed of scattered fascicles of cylindrical hyphae (2.5–5.2 µm diam) that are colorless in mass and frequently septate, thin- to slightly thick-walled; terminal elements poorly differentiated. Contextual hyphae thin- to slightly thick-walled, hyaline, interwoven, frequently branched, commonly anastomosing, 1.5–5.0 diam. Spine tramal hyphae thin-walled toward the tips of the spines, slightly thick-walled toward the core, smooth, hyaline, branching, interwoven, 1.0–3.0 µm diam, sometimes forming intercalary and terminal inflated segments; intercalary inflated segments cylindrical to fusiform, 68–80 × 6.9–13.0 µm; terminal inflated segments long clavate, 40–85 × 9.0–14.0 µm. Hyphidia simple, thin-walled, hyaline, 1.0–2.7 µm diam at the apex, covering basidial cells. Basidia 4-celled, longitudinally septate, multigutullate, barrel-shaped to subglobose, 9.2–12.0 × 8.1–10.6 µm; sterigmata up to 22 × 1.4–2.3 µm, mostly tubiform, usually slightly flaring at apex. Probasidia subglobose to short clavate, 8.8–9.0 × 6.5–7.5 µm, mostly multigutullate. Basidiospores [90/3/2] (5.0–)5.6–6.9(–7.3) × (3.9–)4.3–5.0(–5.5) µm [L_m = 6,2–6.5 µm; W_m = 4.6–4.7 µm; Q = (1.16–)1.23–1.53(–1.66); Q_m = 1.32–1.37], broadly ellipsoid to ellipsoid, rarely elongate, nonamyloid, nondextrinoid, acyanophilous, hyaline, thin-walled, mostly germinating by repetition, sometimes by a germ tube conspicuously long, up to 28 µm long.

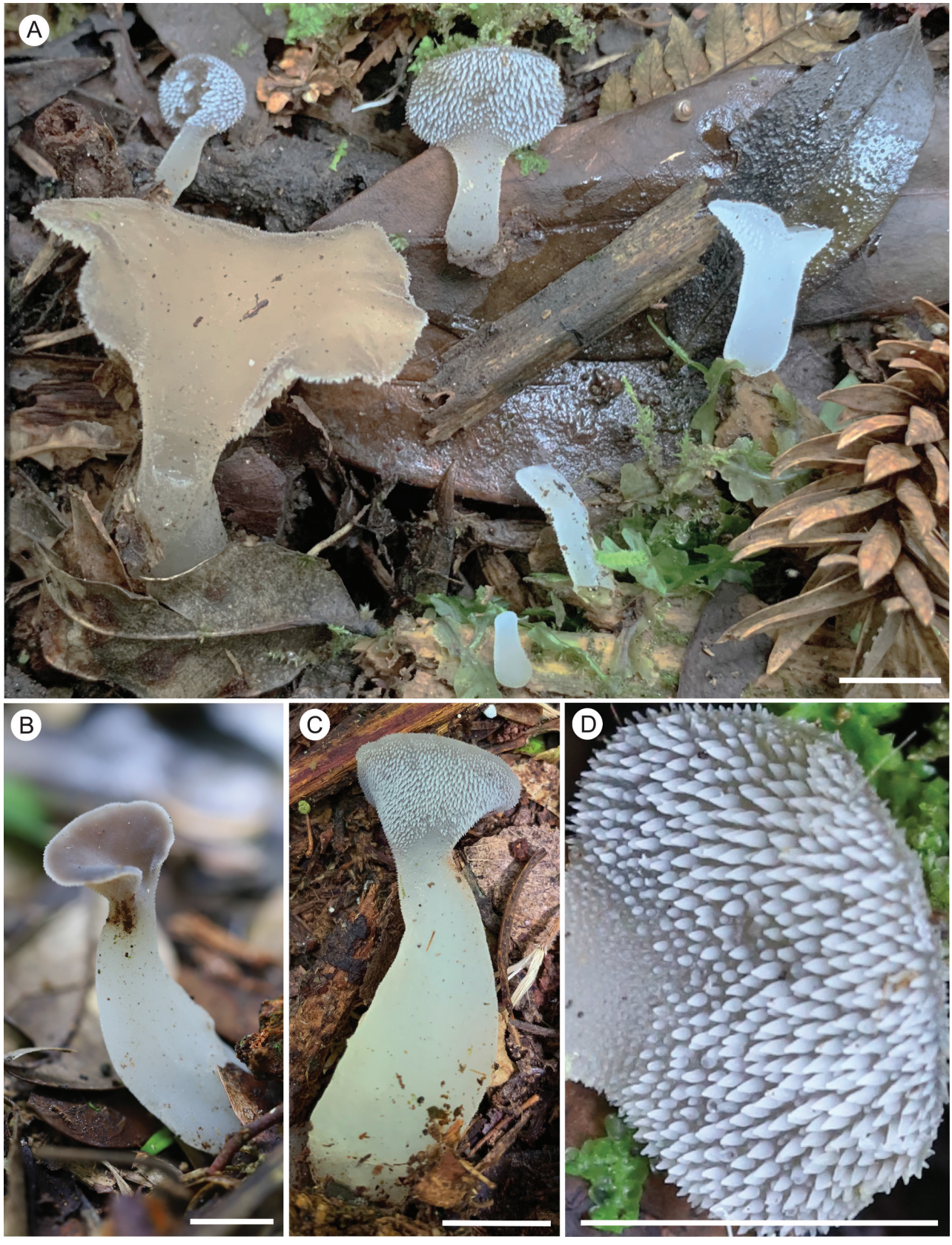

Figure 5. Basidiomata of *Pseudohydnum brasiliense*. A. MPD570 (holotype; FIFUNGI279). B–D. MPD572 (FIFUNGI280). Bars = 10 mm. Photographs: Mariana P. Drewinski.

Figure 6. Basidiomata of *Pseudohydnum brasiliense* with a fawn brown pileus surface. A–B. MIND.Funga0397 (FLOR 71400). C. MPD536 (FIFUNGI281). Bars = 10 mm. Photographs: A–B by MIND.Funga; C by Mariana P. Drewinski.

Habitat and distribution: Growing on decayed wood of *Vernonanthura discolor* in upper montane mixed ombrophilous forest of Southeastern Brazil, at 1600 m a.s.l. Only known for the Brazilian state of Minas Gerais.

Other specimen examined: BRAZIL. MINAS GERAIS: Camanducaia, Serra da Mantiqueira, Monte Verde, from decaying *Vernonanthura discolor* wood, 5 Apr 2023, *C. Coelho-Nascimento & T. Comenale*

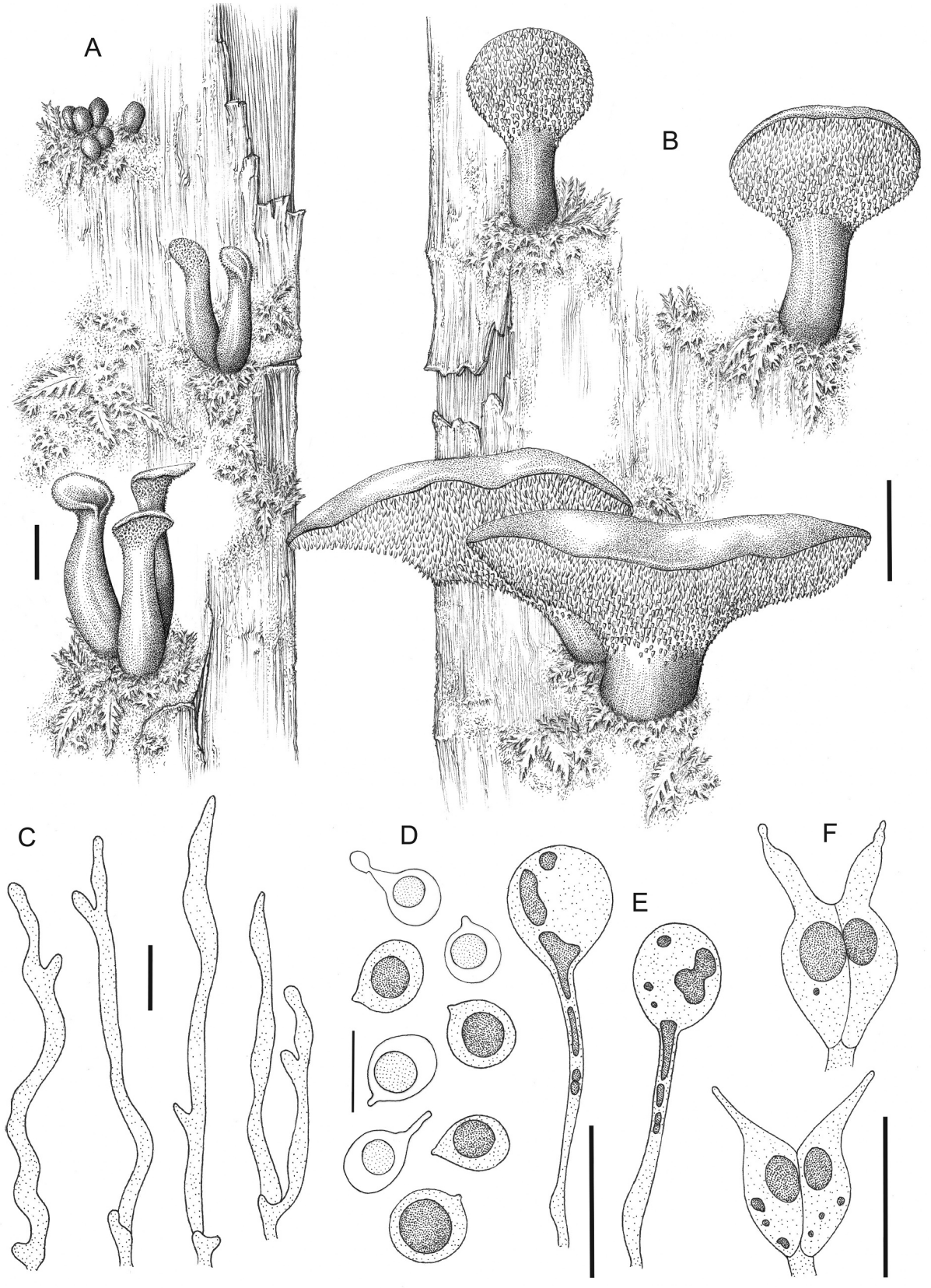

Figure 7. *Pseudohydnum brasiliense* (holotype; MPD570, FIFUNGI279). A. Young basidiomata. B. Mature basidiomata. C. Hyphidia. D. Basidiospores. E. Probasidia. F. Basidia. Bars: A–B = 10 mm; C–F = 10 µm.

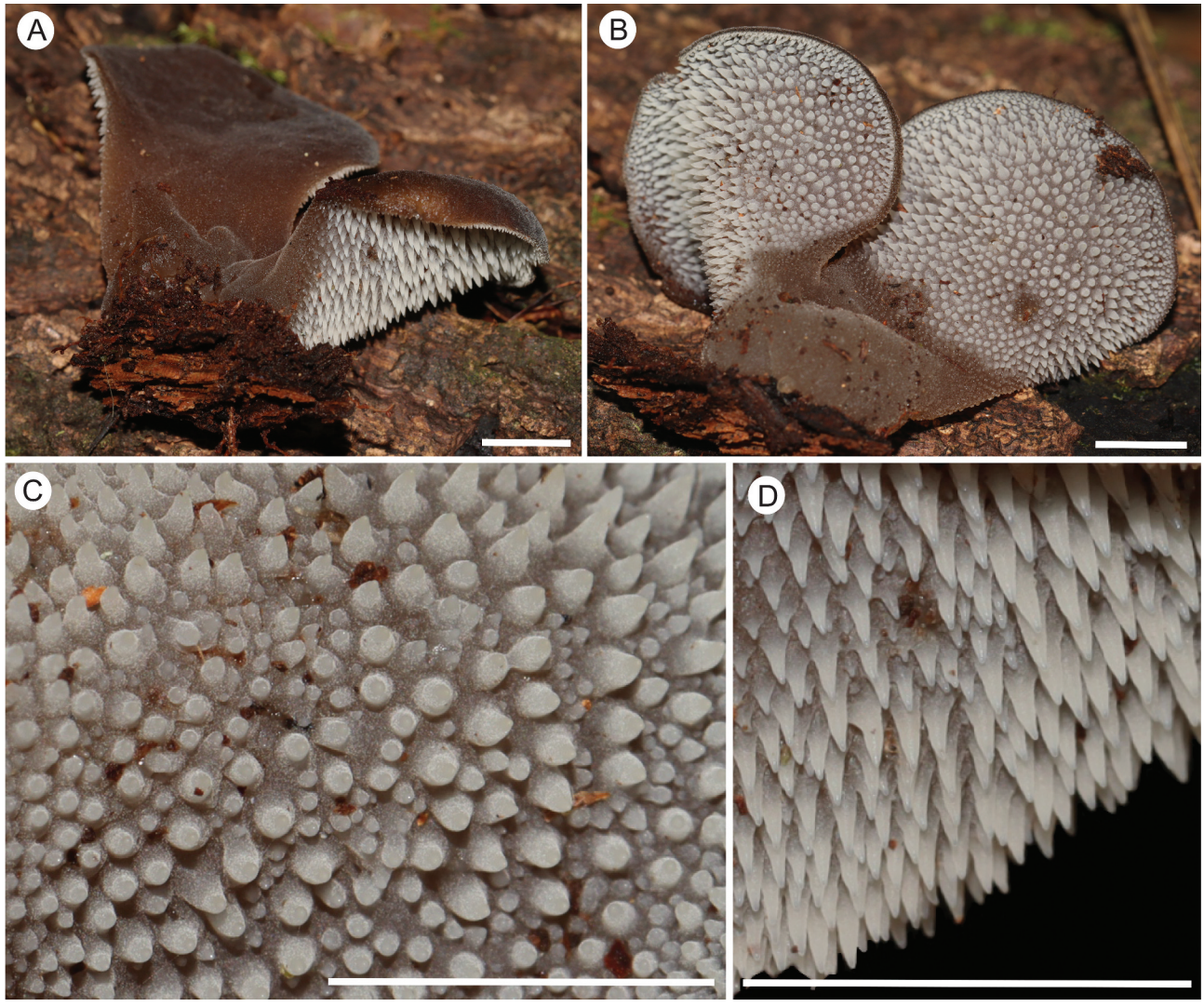

Figure 8. *Pseudohydnum brunneovelutinum* (holotype; AGS118/2022, FIFUNGI283). A–B. Basidiomata. C–D. Detail of the hymenophoral spines. Bars: 10 mm. Photographs: Alexandre G. dos Santos e Silva-Filho.

NCC273 (FIFUNGI293), GenBank: ITS = OR625553, 28S = OR957374.

Notes: In the field, *P. viridimontanum* immediately evokes *P. cupulisnymphae* because of the white to grayish yellow pileus and occasional basidiomata with a rudimentary stipe. Both species also display similar hymenial to subhymenial anatomy. However, *P. cupulisnymphae* differs by its stouter and mostly sessile basidiomata, larger basidiospores (5.8–8 × 5.1–7 µm) with a lower Q_m value (Q_m = 1.10–1.18), clamped generative hyphae, and the absence of cylindrical to fusiform inflated segments in spine trama. Furthermore, *P. viridimontanum* is also different by the ontogenetic configuration in young basidiomata because at first it forms a globose to bell-shaped basidiome that rapidly expands and assumes a mature flabelliform shape. In contrast, all young stages observed throughout *P. cupulisnymphae* collections involve a distinctly funnel-shaped or cupulate configuration.

Phylogenetically, based on the analyses restricted to *Pseudohydnum* species (FIG. 3), *P. viridimontanum* clusters with *P. umbrosum* in a well-supported clade in the BI analysis (lineage 1, BPP = 0.99) that is sister to the lineage around *P. translucens* (lineage 2). This phylogenetic relationship of *P. umbrosum* and *P. translucens* is consistent with the three-marker phylogeny presented by Spirin et al. (2023) but is not consistent with the ITS +28S–based phylogeny presented by them, where the relationship between *P. umbrosum* and the clade around *P. translucens* is not resolved, with both clades placed in a polytomy. From a morphological perspective, *P. viridimontanum* barely resembles *P. umbrosum*. The

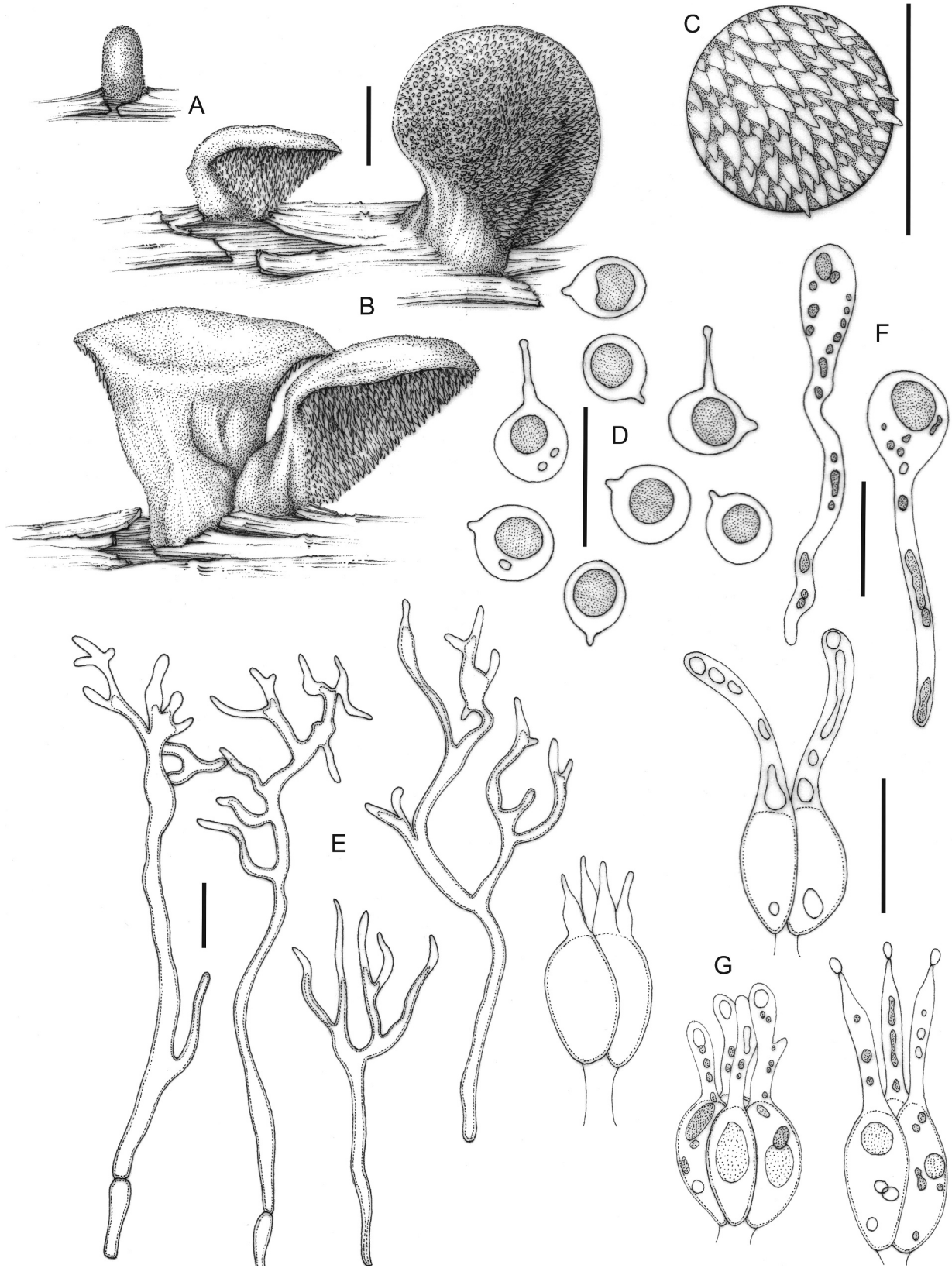

Figure 9. *Pseudohydnum brunneovelutinum* (holotype; AGS118/2022, FIFUNGI283). A. Young basidiomata. B. Mature basidiomata. C. Hymenophoral spines detail. D. Basidiospores. E. Hyphidia. F. Probasidia. G. Basidia. Bars: A–B = 10 µm; C = 5 mm; D–F = 10 µm.

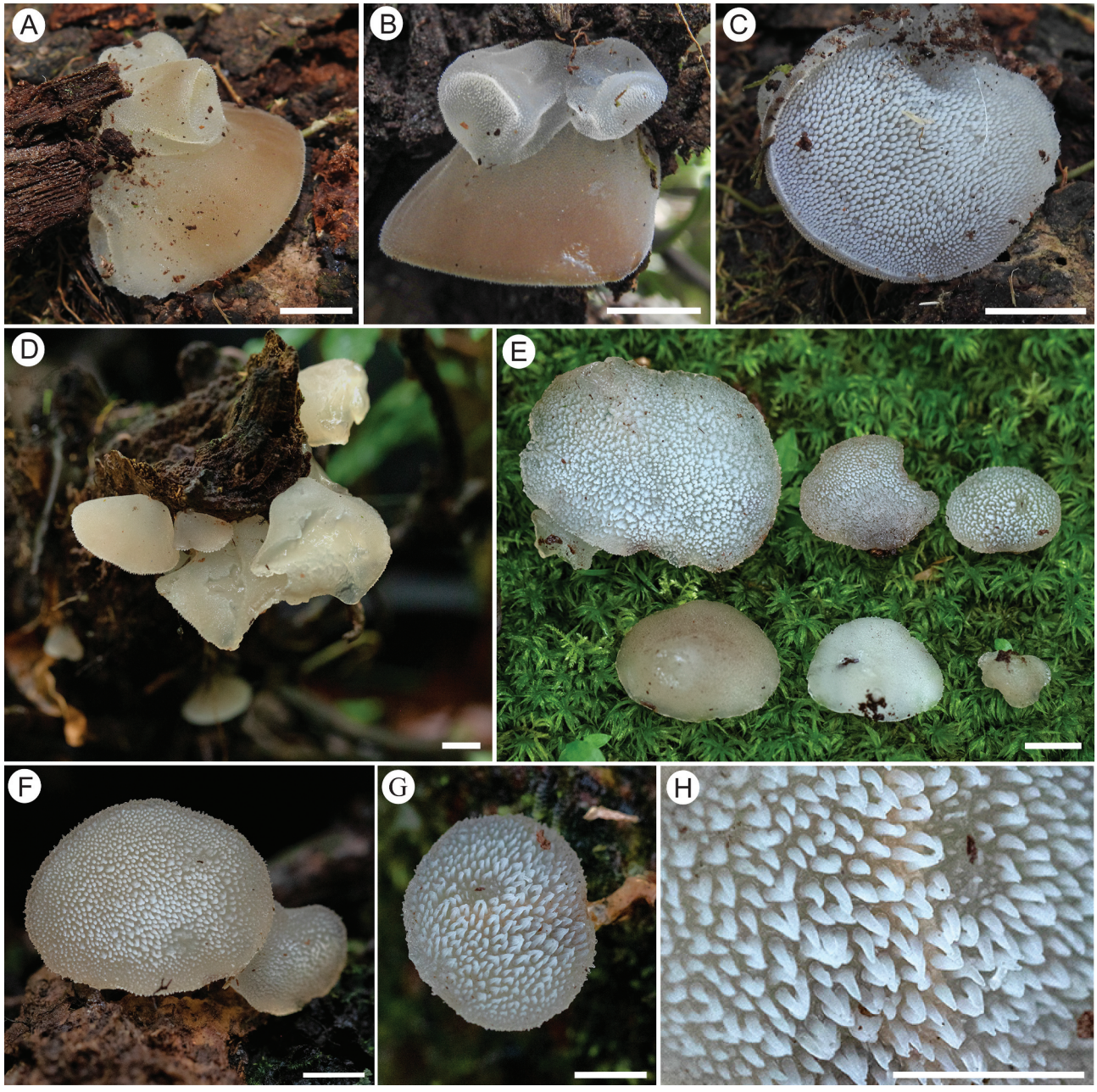

Figure 10. *Pseudohydnum cupulisnymphae*. A–G. Basidiomata. H. Detail of the hymenophoral spines. A–C. MIND.Funga0040 (FLOR71289). D–F. NCC246 (FIFUNGI289). G–H. NCC247 (FIFUNGI 286). Bars = 10 mm. Photographs: A–C by MIND.Funga; D–H by Cristiano C. do Nascimento.

latter is characterized by a dark-colored, fuscous-brown upper surface of pileus, denser hymenophoral spines (5–7 per mm), and larger basidiospores (7.6–9.8 × 6.0–7.1 µm), as well as the lack of cylindrical to fusiform inflated segments in the spine trama (Spirin et al. 2023). *Pseudohydnum umbrosum* also has a very different ecology, growing on decayed conifer wood and so far known only from a boreal forest in the Russian Far East (Spirin et al. 2023).

Pseudohydnum viridimontanum represents an edible wild mushroom that is harvested for consumption during the rainy season, in the type locality and surroundings; it is known as "cat's tongue auricularia" or "white auricularia." The local harvester (key informant) that collected *P. viridimontanum* type specimen (NCC266) reported the frequent consumption of *Pseudohydnum* basidiomata associated with *Vernonanthura discolor* decayed wood. Nevertheless, a systematic multiyear sampling of *Pseudohydnum* specimens in the referred area is necessary in order to confirm whether only *P. viridimontanum* occurs as a frequent species, or whether another similar species,

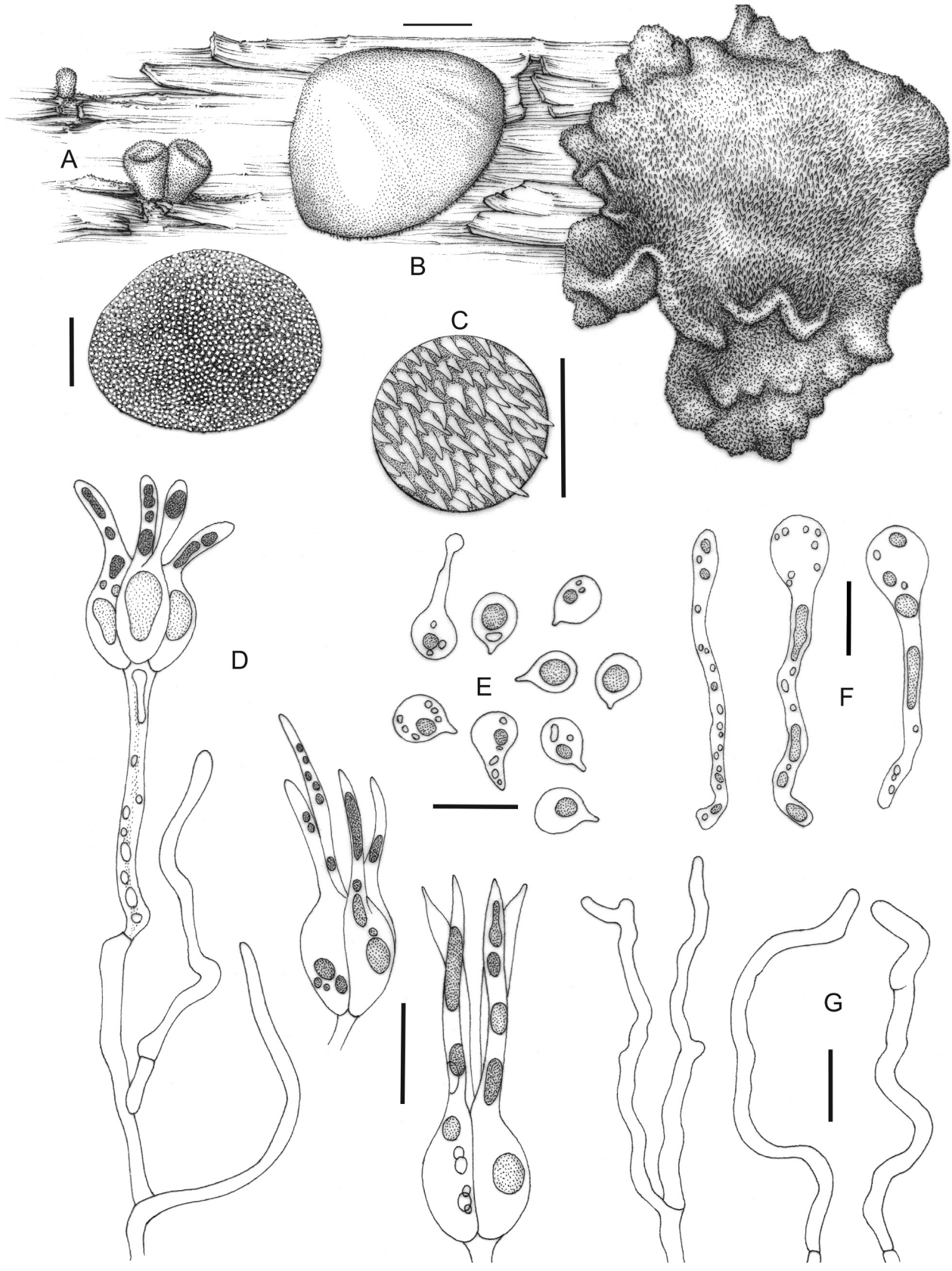

Figure 11. *Pseudohydnum cupulisnymphae* (holotype; NMJ420, FIFUNGI285). A. Young basidiomata. B. Mature basidiomata. C. Hymenophoral spines detail. D. Basidia. E. Basidiospores. F. Probasidia. G. Hyphidia. Bars: A–B = 10 mm; C = 5 mm; D–G = 10 µm.

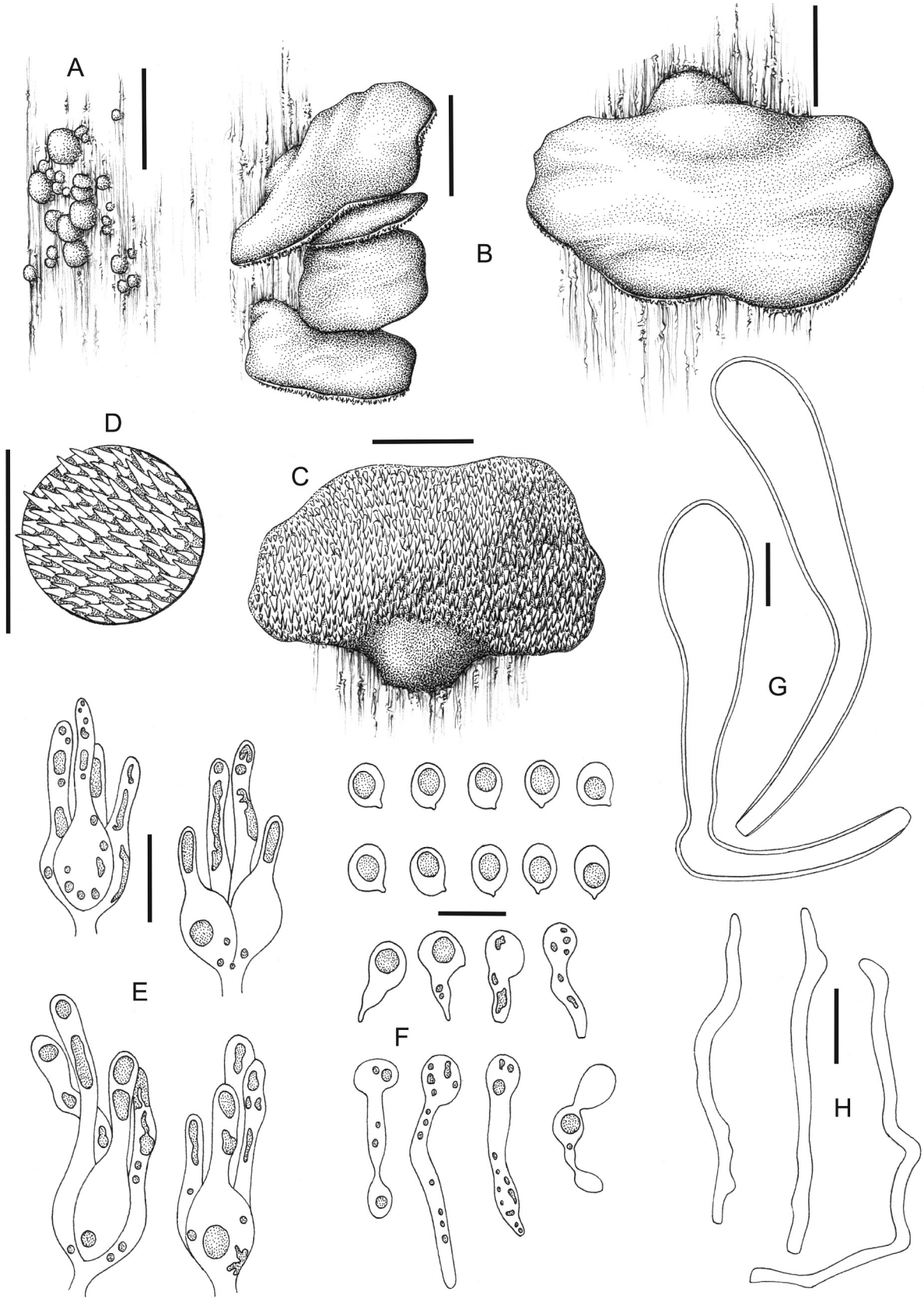

Figure 12. *Pseudohydnum viridimontanum* (holotype; NCC266, FIFUNGI292). A. Primordia and young basidiomata. B. Mature basidiomata, top view. C. Mature basidiomata, underside view. D. Hymenophoral spines detail. E. Basidia. F. Basidiospores. G. Terminal inflated elements from spine trama. H. Hyphidia. Bars: A–C = 10 mm; D = 5 µm; E–G = 10 µm.

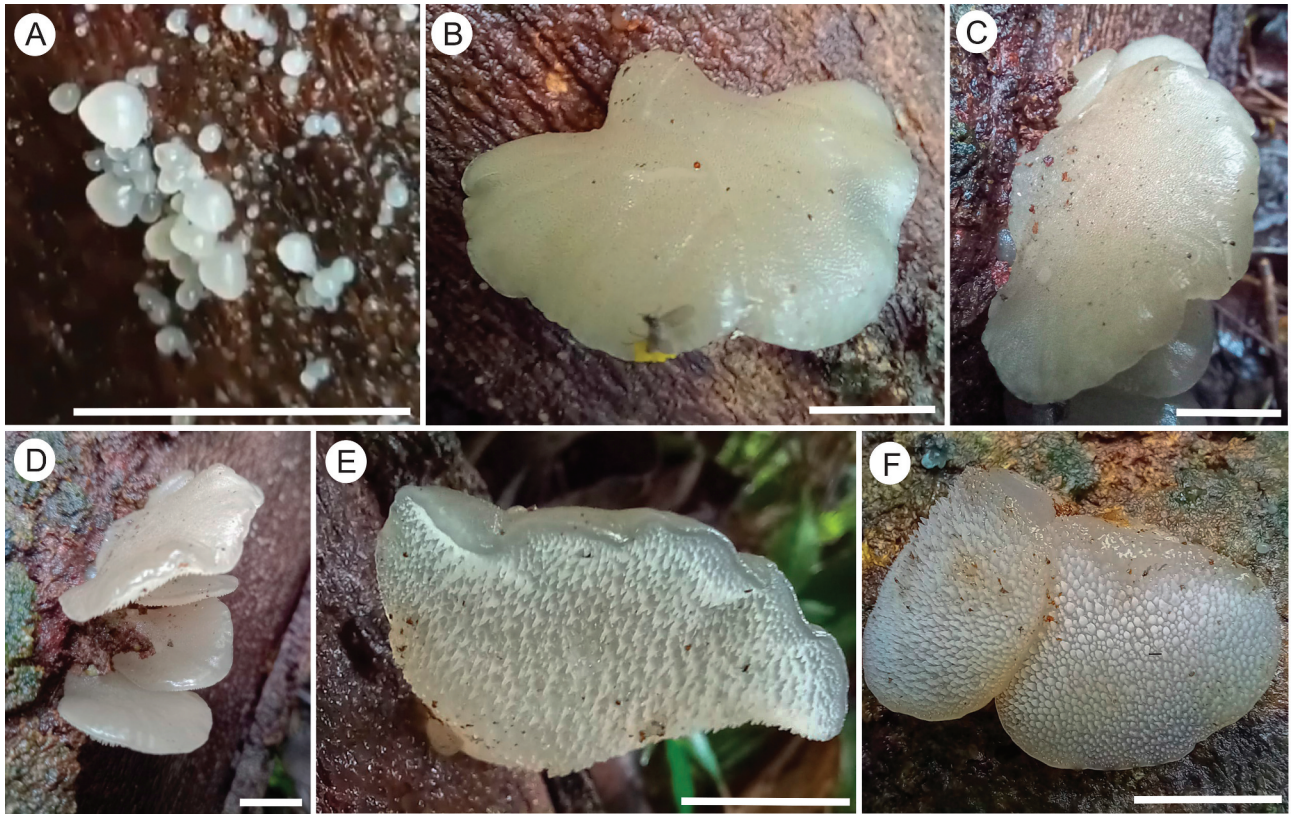

Figure 13. Basidiomata of *Pseudohydnum viridimontanum*. A–C. NCC266 (holotype; FIFUNGI292). D–F. NCC273 (FIFUNGI293). Bars = 10 mm. Photographs: Thiago Comenale.

such as *P. cupulisnymphae*, occurs and is also harvested wild. According to the aforementioned informant, *Pseudohydnum* basidiomata, which grow in large numbers, are collected during every rainy season by him and his family. The preparation for consumption is generally done by quickly sautéing the mushrooms, which are then used as a side dish, usually mixed with salad.

DICHOTOMOUS KEY FOR THE SEVEN CURRENTLY KNOWN TAXA OF *PSEUDOHYDNUM* FROM THE SOUTHERN HEMISPHERE

1. Basidiomata stipitate 2
1′. Basidiomata sessile or rudimentarily stipitate 3
2. Basidiomata confluent; basidiospores 5.5–6.5 × 4.8–5.7 µm ..... *P. totarae*
2′. Basidiomata nonconfluent; basidiospores 6.4–9.7 × 5.4–8.8 µm ..... *P. brasiliense*
3. Pileus surface brown to grayish brown, dark brown, or reddish brown; spines sparse (0.5–1.5 per mm) 4
3′. Pileus surface whitish to grayish, grayish yellow, or pale pinkish orange; spines not sparse (≥2 per mm) 5
4. Basidiomata sessile; simple hyphidia present .... *P. orbiculare*
4′. Basidiomata short to rudimentary stipitate; dendrohyphidia present ..... *P. brunneovelutinum*
5. Basidiospores ≥7.2 × 6.0 µm; on *Eucalyptus* and *Nothofagus* wood; restricted to Tasmania, Australia ..... *P. tasmanicum*
5′. Basidiospores ≤7.0 × 6.0 µm; on angiosperm wood; Neotropical 6
6. Basidiospores subglobose to broadly ellipsoid (Q_m = 1.18); inflated segments in spine trama absent; clamp connections present .... *P. cupulisnymphae*
6′. Basidiospores broadly ellipsoid to ellipsoid (Q_m = 1.37); inflated segments cylindrical to fusiform present in spine trama; clamp connections absent ..... *P. viridimontanum*

DISCUSSION

In the present study, four new species of *Pseudohydnum* were described from Brazil, in addition to 14 species recently introduced by Chen et al. (2020), Zhou et al. (2022, 2023), and Spirin et al. (2023). Even so, the

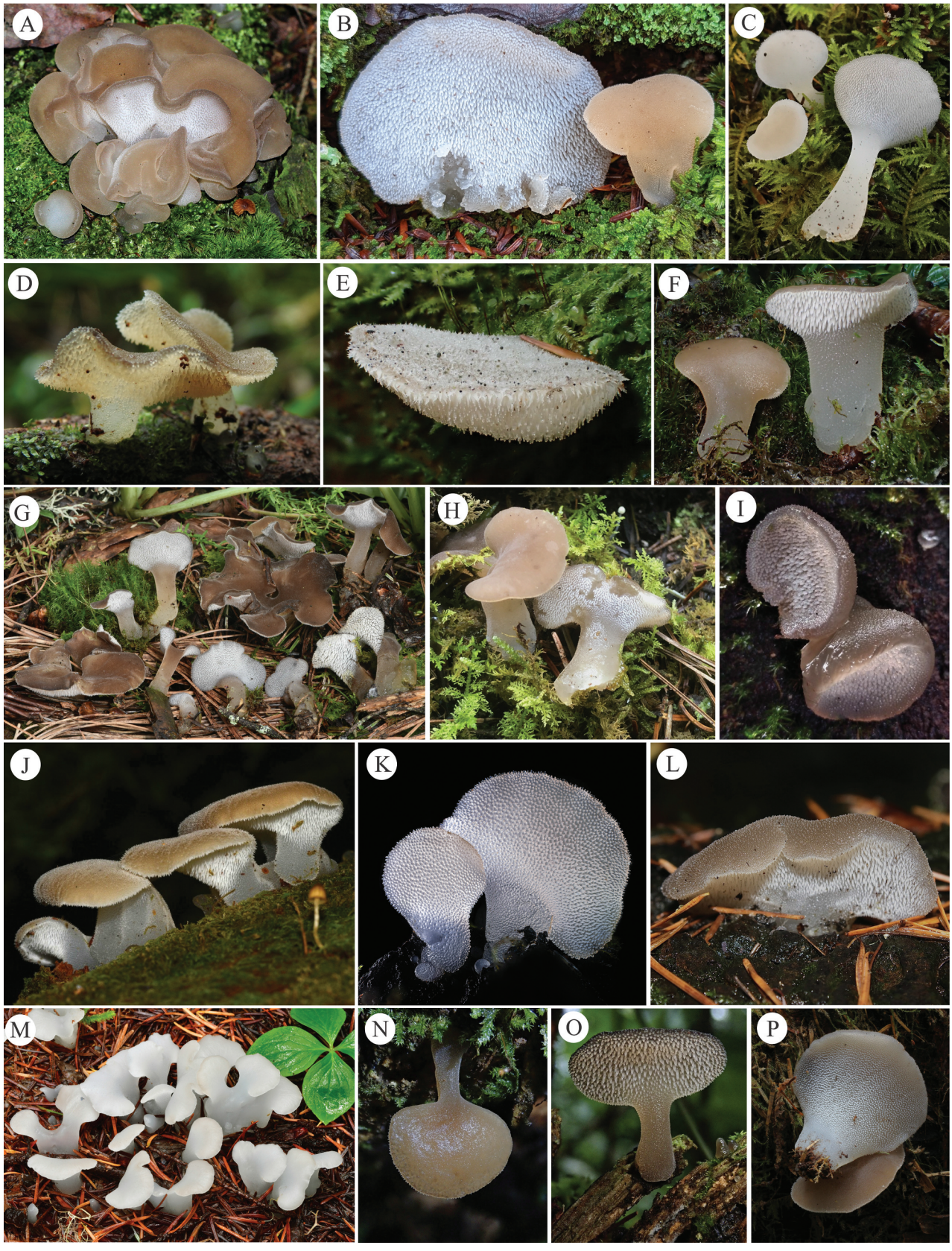

Figure 14. Macromorphological diversity of putatively undescribed *Pseudohydnum* species from the Americas. Data from GBIF/iNaturalist. A–C, J–M, P. USA. D. Brazil. E. Canada. F, N, O. Ecuador. G–H. Mexico. I. Costa Rica. Photographs: A. ©Andrew Khitsun; B. ©Matt Pulk; C. © Elijah Disrude; D. © Felipe Bittencourt; E. © Igor Khomenko; F. © Damon Tighe; G. © Alan Rockefeller; H. © Hugo Basurto; I. © Andrey Loria; J. © Thom Worm; K. © Alan Rockefeller; L. © Colton Veltkamp; M. © Christian Schwarz; N, O. © Nolan Exe; P. © Jonathan Frank.

species diversity in the genus seems to be much higher, especially in the Americas, as we could judge from the unique suite of macromorphological traits observed in a number of *Pseudohydnum* records retrieved from GBIF/iNaturalist platforms (FIG. 14). These records putatively represent undescribed species. Previous Neotropical records of collections identified as *P. gelatinosum* addressed here (viz., Courtecuisse and Lowy 1990; Lowy 1959, 1971; Möller 1895) morphologically fit with *P. cupulisnymphae* and/or *P. brasiliense* and allowed the distribution of both taxa to be expanded to other Neotropical areas. *Hydnum hirneoloides* Berk. & M.A. Curtis from Cuba, an old name associated with *P. gelatinosum*, is distinguished from all specific morphological concepts herein introduced. Further taxonomic studies should carefully reconsider this name when dealing with *Pseudohydnum* collections that display both short-stipitate/sessile basidiomata and a pale brownish pileus (Berkeley and Curtis 1868).

The systematic positioning of the four new described species is robustly supported by morphological and molecular results. The phylogenetic reconstruction based on three markers, ITS, 28S, and *RPB1*, allowed species recognition and also provided evidence for the recognition of eight subgroups corresponding to subclades/lineages (FIGS. 3, 1–8), as well as having been previously retrieved by Spirin et al. (2023). However, some intermediate and deep nodes had no significant statistical support, and there was a lack of resolution between the main recovered lineages. Other questions arising from the presented phylogenetic results will have to be tackled in a wider context with more and geographically wider samples and more loci. These include whether *P. gelatinosum* should be treated as a single transcontinental species or whether to attach any importance to the subtle morphological differences and independent phylogenetic position in order to consider the North American *P. gelatinosum* ssp. *pusillum* to be a distinct taxon. Both ITS+LSU and ITS+LSU+*RPB1* phylogenies clearly support the latter point; the *P. gelatinosum* ssp. *pusillum* sequences closely nest with other North American sequences labeled as "*P. gelatinosum*" and form a well-supported clade in the ML analysis (BS = 87%) (FIG. 3). Moreover, two collections also named "*P. gelatinosum*" were inferred as a well-supported clade (BPP = 1, BS = 100%) sister to *P. himalayanum* and which most likely represents an undescribed species.

Morphologically, the delimitation and recognition of the species described in detail here are unambiguous, although we assume that the limited availability of anatomical characters and the species' occasional high variability may represent an obstacle for morphological definition of *Pseudohydnum* species, mainly in terms of macromorphology. The only microscopic traits useful for species differentiation are those associated with the contextual and tramal hyphae, hyphidia, and basidiospores. The presence of clamp connections seems to be a common trait throughout all species of *Pseudohydnum* known so far. It was a difficult character to assess in some collections studied, especially in parts other than the hymenium. Remarkably, accurate analyses of all *P. viridimontanum* basidiomata failed to spot any clamp connections, showing that species are able to proliferate and reproduce without the necessity of clamp formation that putatively occurs in the *Pseudohydnum* lineage.

To provide a comprehensive framework for the *Pseudohydnum* species delimitation, we also propose to pay attention to some diagnostic macromorphological and ontogenetic traits. Macromorphologically, we regard the pileal surface, basidioma color(s), and, especially, the presence/absence of stipe of paramount taxonomic importance. Concerning the latter trait, basidiomata can range from distinctly stipitate to sessile. It is important to highlight that some species (e.g., *P. cupulisnymphae*) have mostly sessile basidiomata, producing occasional basidiomata with a rudimentary stipe (pseudostipitate), whereas other species (e.g., *P. viridimontanum* and *P. gelatinosum*) are mostly short-stipitate but sometimes exhibit pseudostipitate or sessile basidiomata (Spirin et al. 2023). In contrast, some species seem to consistently produce stipitate basidiomata with either a short (e.g., *P. brunneovelutinum*) or a well-developed (e.g., *P. brasiliense*) stipe.

Ontogenetic characters are more difficult to assess because it is necessary to observe the basidiomata in their sequential developmental stages, from primordia to maturity. These traits were valuable to distinguish the species of *Pseudohydnum* described here. Specimens of *P. brasiliense*, for instance, always produce noncoalescent young basidiomata with a pseudoinfundibuliform pileus and a slightly inflated stipe (FIG. 5C), whereas the young stages of *P. cupulisnymphae* exhibit a cup-shaped pileus, with basidiomata often growing aggregated and forming a coalescent base (FIG. 9B). On the other hand, development in *P. brunneovelutinum* and *P. viridimontanum* is somewhat more direct, with intermediate stages that clearly resemble the basidiomata at maturity.

Species of *Pseudohydnum* occur all over the world, although the knowledge of species diversity and distribution remains rather fragmentary. Most of the known described species are from temperate to boreal Eurasia, suggesting that further studies are necessary

in order to recognize *Pseudohydnum* diversity worldwide, especially in Neotropical habitats with high conservation value. Although the present study could be limited with regard to collecting sites and the number of collections studied, four new species from Brazil are described, and this provides an important step in the understanding of *Pseudohydnum* in the Neotropics and a basis for further development.

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