Denis Augusto Zabin

Diversidade e crescimento micelial *in vitro* de cogumelos comestíveis silvestres *Favolus* spp. (*Polyporaceae*, *Agaricomycetes*)

Dissertação apresentada ao Instituto de Pesquisas Ambientais, da Secretaria de Infraestrutura e Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de MESTRE em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Avasculares e Fungos em Análises Ambientais.

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À toda gente estranha deste mundo que decidiu estudar esses organismos tão curiosos, peculiares, complicados e fascinantes que são os fungos!

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RESUMO

Favolus é um gênero monofilético de políporos distribuídos mundialmente que causam a decomposição do tipo podridão branca da madeira morta de diversas espécies vegetais, e cujos representantes podem ser caracterizados macromorfologicamente pelos basidiomas carnosos, lateralmente estipitados e, em sua maioria, pelo himenóforo com poros angulares e radialmente alongados. Além de sua importância ecológica, o gênero é importante economicamente, sendo fonte de alimento, como cogumelo comestível silvestre, para diferentes comunidades tradicionais mundo afora, dentre elas, o povo Yanomami da Amazônia brasileira, que faz uso das espécies *Favolus brasiliensis*, *F. radiatifibrillosus* e *F. yanomamii* em sua dieta. Considerando a diversidade ainda pouco explorada dos fungos no Brasil e o potencial alimentício e para o cultivo comercial de espécies de *Favolus*, coletas foram realizadas em áreas de Mata Atlântica do Sudeste brasileiro para obtenção de novos espécimes e novos isolados miceliais visando a elucidação da diversidade do gênero na região e a realização dos testes de crescimento micelial *in vitro*. Análises morfológicas dos materiais coletados e análises filogenéticas multigênicas (ITS, nucLSU, *TEF1* e *RPB1*) foram realizadas visando a investigação do "complexo de espécies" *F. brasiliensis* e a identificação e o posicionamento filogenético dos materiais coletados e dos isolados obtidos. Foram geradas 87 novas sequências de *Favolus* spp. (35 ITS, 25 nucLSU, 19 *TEF1* e 8 *RPB1*). Duas espécies novas são propostas com base em análises morfológicas e filogenéticas moleculares: *Favolus brunneofibrillosus*, filogeneticamente e morfologicamente próxima a *F. yanomamii*, e *F. glaucovelutinus*, que forma um clado irmão a *F. rugulosus*. Além disso, com base em sequências de tipos depositadas no GenBank, foi possível elucidar o posicionamento filogenético de *F. elongoporus* e propor a combinação de *Polyporus laetiporoides* em *Favolus*. Culturas puras de *Favolus brasiliensis*, *F. brunneofibrillosus* e *F. rugulosus* foram submetidas aos testes para avaliação da velocidade de crescimento micelial e produção de biomassa *in vitro* em diferentes temperaturas (20 ºC, 25 ºC, 30 ºC e 35 ºC) e meios de cultura [Batata Dextrose Ágar (BDA), Malte Levedura Peptona Ágar (MLPA), Levedura Glicose Ágar (LGA) e Ágar Soja]. As melhores temperaturas para o crescimento micelial e produção de biomassa para os isolados estudados foram 25 °C ou 30 °C, e todos foram capazes de crescer em todos os meios de cultura testados. O isolado de *F. brasiliensis* CCIBt 4770 obteve o melhor crescimento micelial em geral, com as melhores combinações de meio de cultura e temperatura sendo MLPA ou LGA a 25 ºC, e o isolado pode ser priorizado para futuros estudos de domesticação. Para o isolado CCIBt 4769 de *F. brunneofibrillosus*, as combinações relacionadas ao melhor crescimento foram MLPA ou BDA a 25 ºC ou 30 ºC. Finalmente, o isolado CCIBt 4784 de *F. rugulosus* apresentou seu melhor desempenho quando cultivado em meio Ágar Soja a 30 ºC. Os resultados obtidos nesse trabalho contribuíram com o entendimento da diversidade do gênero *Favolus* no Brasil e poderão servir como base para próximos estudos de domesticação para espécies comestíveis do gênero.

Palavras-chave: cultivo de cogumelos; filogenia; Mata Atlântica; *Polyporaceae*; *Favolus brasiliensis; Favolus rugulosus*; taxonomia.

ABSTRACT

Favolus is a monophyletic genus of polypores with a worldwide distribution that causes white rot decomposition of dead wood of various plant species and that can be characterized macromorphologically by the fleshy and laterally stipitate basidiomata, with mostly angular and radially elongated pores. In addition to its ecological importance, the genus is economically important, being a source of food, as a wild edible mushroom for different traditional communities around the world, among them, the Yanomami people of the Brazilian Amazon, who make use of the species *Favolus brasiliensis*, *F. radiatifibrillosus* and *F. yanomami* in their diet. Considering the still little-explored fungal diversity in Brazil and the potential as food and for the commercial cultivation of *Favolus* species, collections were carried out in areas of the Atlantic Forest in southeastern Brazil to obtain new specimens and new isolates, aiming to elucidate the diversity of the genus in the region and for testing the effect of different growth factors on the *in vitro* mycelium growth. Morphological analyses of the collected materials and multigenic phylogenetic analyses (ITS, nucLSU, *TEF1* and *RPB1*) were carried out. Eighty-seven new sequences from *Favolus* spp. were generated (35 ITS, 25 nucLSU, 19 *TEF1* and 8 *RPB1*). Two new species are proposed based on morphology and multigene phylogenies: *Favolus brunneofibrillosus*, which is phylogenetically and morphologically close to *F. yanomamii*, and *F. glaucovelutinus*, which forms a sister clade to *F. rugulosus*. Furthermore, based on type sequences deposited in GenBank, it was possible to elucidate the phylogenetic positioning of *F. elongoporus* and the combination of *Polyporus laetiporoides* in *Favolus* is proposed. Pure cultures of *Favolus brasiliensis*, *F. rugulosus* and *F. brunneofibrillosus* were tested to evaluate the mycelial growth velocity and biomass production at different temperatures (20 ºC, 25 ºC, 30 ºC and 35 ºC) and solid culture media (Potato Dextrose Agar, Malt Yeast Peptone Agar, Yeast Glucose Agar and Soy Agar). The best temperatures for the mycelium growth and biomass production for the studied strains were either 25 °C or 30 °C, and all the strains were able to grow on all tested solid culture media. The *F. brasiliensis* wild strain CCIBt 4770 achieved the best mycelium growth overall, with the best combinations of culture media and temperature being either MYPA or YGA at 25 ºC, and this strain should be prioritized for future domestication studies. For the *F. brunneofibrillosus* wild strain CCIBt 4769, the combinations related to the best growth were either MYPA or PDA at 25 ºC or 30 ºC. Finally, the *F. rugulosus* wild strain CCIBt 4784 performed at its best when growing on the SOY medium at 30 ºC. The results obtained in this work contributed to the understanding of the diversity of the genus *Favolus* in Brazil and may serve as a basis for future domestication studies for the edible species of the genus.

Keywords: Atlantic Forest; mushroom cultivation; phylogeny; taxonomy; *Favolus brasiliensis*; *Favolus rugulosus*

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APRESENTAÇÃO

Este trabalho constitui um estudo sistemático e de avaliação de fatores relacionados ao crescimento micelial *in vitro* de espécies e isolados do gênero *Favolus* (Polyporaceae, Agaricomycetes) obtidos em remanescentes de Mata Atlântica do Sudeste brasileiro.

Na Introdução, é apresentada a fundamentação teórica para este trabalho, que incluem dados atualizados referentes à diversidade estimada e os números de espécies conhecidas de fungos no mundo e no Brasil, assim como a importância e o panorama global e nacional sobre o cultivo de cogumelos comestíveis e o histórico dos estudos taxonômicos e de cultivo sobre o gênero *Favolus*, até o momento.

Os resultados deste trabalho são apresentados em dois capítulos, que correspondem a manuscritos a serem submetidos para publicação e que incluem a parte dos procedimentos metodológicos de cada estudo.

O manuscrito correspondente ao Capítulo I traz a descrição de duas novas espécies de *Favolus*, além de uma investigação do complexo de espécies *F. brasiliensis*, com base em estudos morfológicos e filogenéticos moleculares utilizando quatro marcadores (ITS, nucLSU, *TEF1* e *RPB1*), e a elucidação do posicionamento filogenético de *F. elongoporus* e de uma coleção morfologicamente próxima a *F. radiatifibrillosus*. O manuscrito segue a formatação recomendada pela revista *Mycological Progress*, onde será submetido.

O manuscrito do Capítulo II, por sua vez, corresponde aos estudos de crescimento micelial *in vitro* de quatro isolados de *Favolus*: dois isolados de *F. brasiliensis*, um isolado de *F. brunneofibrillosus* e um isolado de *F. rugulosus.* Neste capítulo, são apresentados os resultados da avaliação do efeito de diferentes temperaturas (20 ºC, 25 ºC, 30 ºC e 35 ºC) e meios de cultura sólidos (Batata Dextrose Ágar, Malte Levedura Peptona Ágar, Levedura Glicose Ágar e Ágar Soja) sobre a velocidade de crescimento micelial e a produção de biomassa. O manuscrito segue a formatação recomendada pela revista *Mycoscience*.

Os epítetos das espécies novas citadas neste trabalho serão efetivamente e validamente publicadas quando do aceite e publicação efetiva dos artigos, de modo que esta publicação não deve ser considerada para efeitos taxonômicos de acordo com os artigos 29–31 do Código Internacional de Nomenclatura de Algas, Fungos e Plantas de 2018. As sequências de DNA obtidas serão depositadas em sua totalidade no GenBank quando da submissão dos artigos para publicação.

INTRODUÇÃO GERAL

Os fungos são organismos eucarióticos, unicelulares ou multicelulares e heterótrofos com uma grande diversidade de formas e estratégias de vida e que exercem essenciais funções ecossistêmicas (Dix 2012, Webster & Weber, 2007). Como organismos heterótrofos, os fungos não produzem sua própria fonte de energia e, portanto, dependem principalmente da digestão enzimática extracelular de diferentes substratos orgânicos complexos para aquisição de nutrientes e produção de energia (Richards & Talbot, 2013). Dentre as funções ecossistêmicas exercidas pelos fungos, a participação dos fungos sapróbios está entre uma das mais importantes, atuando na decomposição da matéria orgânica morta como forma de aquisição energética e participando dos ciclos biogeoquímicos a partir da ciclagem de nutrientes nas diferentes esferas dos ecossistemas terrestres e aquáticos (Niego *et al*. 2023, Schöll *et al*. 2008, de Mattos-Shipley *et al*. 2016). Dentre outras estratégias para aquisição energética pelos fungos estão as associações simbióticas com diferentes organismos, como as raízes de plantas fotossintetizantes ou algas verdes e cianobactérias, conhecidas como micorrizas e liquens, respectivamente (Kohler *et al*. 2015; Asplund & Wardle, 2017). Outros fungos, além de atuarem na ciclagem de nutrientes, podem participar de teias alimentares como parasitas ou predadores, participando do controle populacional de diferentes organismos (Vega *et al*. 2009, Barron, 2003).

Quanto ao número de espécies, são formalmente reconhecidas, até o momento, cerca de150 mil espécies de fungos (Lücking *et al.* 2021a). No entanto, estimativas globais apontam para um número entre 2,2 e 12 milhões de espécies de fungos no mundo (Blackwell, 2011; Hawksworth & Lücking, 2017, Wu *et al*. 2019). Tais estimativas levam em consideração extrapolações da razão entre o número de espécies de fungos e plantas (entre 6:1 e 10:1), espécies ainda não descritas em *hotspots* de biodiversidade e em nichos ainda pouco explorados, espécies crípticas em complexos de espécies e materiais em herbários a serem revisados, bem como os avanços nas técnicas de biologia molecular e o grande número de dados provenientes do sequenciamento de amostras ambientais não cultivadas (Lücking *et al.* 2021a).

No Brasil, trabalhos de revisão sobre a diversidade de fungos indicam um número de 13.950 espécies citadas para o país (Gaya *et al.* 2022), com a Mata Atlântica sendo o domínio fitogeográfico com o maior número de espécies citadas (Maia *et al*. 2015). Apesar disso, é estimado que entre 9,5% (Lewinsohn & Prado, 2005) e 14% (Forzza *et al*. 2010) da diversidade global estimada de fungos deva ocorrer no Brasil, sendo, portanto, o número de 13.950 espécies

bastante aquém da riqueza esperada. Essa diversidade ainda desconhecida pode ofuscar uma riqueza de espécies com diversos potenciais e aplicações também desconhecidos e inexplorados, como biotecnológicos, industriais e farmacológicos, além de espécies comestíveis com potencial alimentício e para o cultivo comercial, como os cogumelos (Niego *et al.* 2021, 2023; Hyde *et al*. 2019).

Os cogumelos, sejam eles cultivados ou coletados em ambientes silvestres, se referem às estruturas reprodutivas sexuadas macroscópicas dos fungos, chamados de esporomas, e que são típicos de representantes da classe Agaricomycetes, filo Basidiomycota (Alexopoulos *et al*. 1996). Eles possuem grande valor como fonte de renda e como alimento, além de uma grande importância sociocultural (Pérez-Moreno *et al*. 2020, 2021). Os registros históricos do consumo de cogumelos remontam há pelo menos 18.700 anos na Espanha, no paleolítico superior (Power *et al*. 2015, Strauss *et al*. 2015) e entre 5000 e 6000 anos na China (Chang, 2006). Além desse longo histórico com as sociedades humanas, atualmente, o consumo e o interesse pelos cogumelos vêm crescendo significativamente (Li *et al*. 2021). Possíveis motivos para esse interesse incluem a crescente demanda por novos produtos alimentícios, a busca por novas e alternativas fontes de renda em centros urbanos e comunidades rurais (Li *et al*. 2021), e também o reconhecido valor gastronômico dos cogumelos comestíveis, onde os cogumelos comestíveis silvestres têm ganhado destaque com o resgate e valorização de usos e práticas ancestrais por comunidades tradicionais promovendo novas experiências sensoriais e gastronômicas (Fusté-Forné, 2019). Os cogumelos são valorizados pelos seus aromas, texturas e sabores únicos, sendo fontes de diferentes compostos não-voláteis, como aminoácidos livres, nucleotídeos e peptídeos, responsáveis pelo característico sabor umami, assim como compostos voláteis, como aldeídos, cetonas e ésteres, relacionados aos seus aromas distintos (Jiang *et al*. 2023). Os cogumelos comestíveis são também estimados pelo seu valor nutricional, sendo uma fonte, principalmente, de fibras, proteínas, minerais e vitaminas (Kalač, 2013) e pela grande diversidade de compostos bioativos, como beta-glucanas, compostos fenólicos, esteróis e triterpenos, com diferentes potenciais terapêuticos como antioxidante, anti-inflamatório, neuro e imunomoduladores, dentre outras atividades (Ma *et al*. 2018).

Quanto à diversidade de cogumelos, são estimadas entre 14 e 22 mil espécies (Hawksworth, 2001, Mueller *et al*. 2007), sendo 2.189 consideradas comestíveis (Li *et al*. 2021). No entanto, apenas cerca de 100 espécies são domesticadas no mundo e 60 delas são cultivadas comercialmente como alimento e consumidas mais amplamente (Li & Xu, 2022). Ainda assim, apenas cinco gêneros constituem cerca de 85% do mercado global de cogumelos comestíveis: *Lentinula* Earle como o principal gênero cultivado, com *Lentinula edodes* (Berk.)

Pegler sendo a única espécie cultivada comercialmente do gênero, contribuindo com cerca de 22% da produção mundial, seguido por *Pleurotus* (Fr.) P. Kumm. (19%), *Auricularia* Bull. (17%), *Agaricus* L. (15%) e *Flammulina* P. Kumm. (11%) (Royse et al. 2017).

No Brasil, vem crescendo o interesse pelos cogumelos comestíveis silvestres e cultivados, assim como o mercado nacional de cogumelos. As regiões sul e sudeste concentram a maior parte da produção de cogumelos comestíveis do país, sendo o estado de São Paulo o maior produtor, com cerca de 505 produtores distribuídos no estado (Gomes et al. 2016), seguido pelo estado do Paraná, que juntos constituem 90% da produção nacional (Sanchéz et al. 2018). Os dados mais recentes apontam para uma produção anual de 15.696 toneladas no Brasil, correspondendo a cerca de 2,35% da produção total das Américas (Sanchéz et al. 2018). Essa produção total está concentrada no cultivo de três espécies, *Pleurotus ostreatus* (Jacq.) P. Kumm. (48%), *Agaricus bisporus* (J.E. Lange) Imbach (33%) e *Lentinula edodes* (13%), e os restantes 6% compostos por outras espécies, como *Agaricus subrufescens* Peck., *Pleurotus djamor* (Rumph. ex Fr.) Boedijn, *P. cornucopiae* (Paulet) Quél., *P. eryngii* (DC.) Quél., entre outras (Sanchéz et al. 2018).

Os isolados utilizados para o cultivo comercial dessas principais espécies em áreas tropicais são, em sua maioria, originados de regiões temperadas (Stamets, 2000), o que requer a otimização das condições climáticas durante o cultivo e que, por sua vez, pode acarretar um custo maior para os fungicultores e pode tornar seu cultivo em larga escala em climas com temperaturas mais quentes insustentável (Vargas-Isla & Ishikawa, 2008). A maioria dos isolados de cogumelos de regiões tropicais, no entanto, podem se desenvolver rapidamente e formar basidiomas a 25 ºC ou mesmo em temperaturas mais elevadas (Thawthong *et al*. 2014) e, assim, pode ser produzida de forma mais eficiente nessas regiões do que as espécies de regiões de clima temperado. Cogumelos comestíveis de regiões tropicais também podem ser cultivados em resíduos agroindustriais prontamente disponíveis e baratos, como serragem, bagaço de cana-de-açúcar, palha de arroz e vários outros resíduos lignocelulósicos (Kumla *et al*. 2020, Omarini *et al*. 2009). Ademais, o cultivo de cogumelos também pode ser incluído em esquemas circulares de produção, possibilitando, além da conversão de resíduos agroindustriais baratos em alimentos nutritivos de alta qualidade, a utilização dos substratos exauridos para o cultivo de novos cogumelos, assim como para a alimentação animal, o cultivo de plantas como composto e a produção de biomateriais e biocombustíveis (Grimm & Wösten, 2018, Zied *et al*. 2020).

Os cogumelos possuem uma enorme diversidade de formas e preferências ecológicas, no entanto, não possuem um conjunto de caracteres morfológicos, sensoriais ou ecológicos

específicos em comum que sinalizam sua comestibilidade ou toxicidade, tampouco algum teste generalizado que permita essa distinção. A tentativa e erro, por sua vez, baseada na ingestão de pequenas porções de potenciais alimentos para verificação da palatabilidade e ausência de efeitos adversos, é apontada como o principal método que permitiu o reconhecimento de espécies comestíveis com maior sucesso ao longo da história (Li et al. 2021). Dessa forma, o conhecimento e o registro etnomicológico de comunidades tradicionais rurais e indígenas se mostram como uma excelente fonte acerca da comestibilidade de espécies de cogumelos (Li *et al.* 2021).

A etnomicologia é a área de estudo que envolve as relações, os usos e os conhecimentos de comunidades tradicionais quanto aos fungos (Yamin-Pasternak, 2011). Valentina Wasson e Gordon Wasson, considerados pioneiros da etnomicologia, propuseram e recontextualizaram os termos micofobia e micofilia como parâmetros para caracterizar as relações, valores, significados e afinidades históricas das sociedades em relação aos fungos (Wasson & Wasson, 1957). Sociedades consideradas micófilas possuiriam um histórico de afinidade e presença dos fungos em suas atividades, seja em usos ritualísticos, medicinais e alimentícios; enquanto sociedades micófobas seriam aquelas que possuem aversão ou desprezo generalizado aos fungos (Wasson & Wasson, 1957).

No Brasil, as comunidades tradicionais indígenas foram inicialmente consideradas micófobas (Fidalgo, 1976). Foi apenas nas décadas de 70 e 80 do século passado que Prance (1972, 1973), Fidalgo e Prance (1976), Fidalgo e Hirata (1979) e Fidalgo e Prance (1984), em seus trabalhos pioneiros de etnomicologia nas comunidades indígenas brasileiras, relataram pela primeira vez a utilização de diferentes espécies de cogumelos na dieta dos indígenas Yanomami da região amazônica entre a Venezuela e o Brasil. Os indígenas Yanomami do grupo Sanöma utilizam diferentes nomes para as espécies de cogumelos comestíveis, ou "*ana amopö*", como são chamados por eles, demonstrando um elevado grau de distinção taxonômica entre as espécies utilizadas, além de conhecimento acerca da ecologia e fenologia das espécies que participam de sua dieta (Sanuma *et al.* 2016). Dentre as espécies de cogumelos presentes na dieta dos Yanomami estão três espécies do gênero *Favolus* Fr.: *Favolus brasiliensis* (Fr.) Fr., *F. radiatifibrillosus* Palacio & R.M. Silveira e *F. yanomamii* Palacio & Menolli, ou *waikasö amo, ara amokë* e *atapa amo,* como são chamados pelos indígenas, respectivamente (Sanuma *et al.* 2016; Palacio *et al.* 2021). *Favolus yanomamii* foi descrita posteriormente à publicação do livro de Sanuma *et al*. (2016) e seu nome é uma homenagem ao consumo dos basidiomas dessa espécie pelos Yanomami, que no livro haviam sido identificados como *Polyporus philippinensis* Berk., uma espécie descrita para as Filipinas e distribuída na Ásia, embora, já

indicando que poderia corresponder a uma espécie distinta de *Favolus* (Palacio *et al*. 2021). Cogumelos do gênero *Favolus* coletados para o consumo pelos indígenas Yanomami costumam ser tradicionalmente preparados assados na brasa, embrulhados em folha de bananeira, ou cozidos em água com sal e pimenta, e são consumidos acompanhados de banana verde assada e beiju, que é uma massa semelhante a uma panqueca, preparada a partir da fécula de mandioca (Sanuma *et al*. 2016). Além dessas espécies consumidas pelo povo Yanomami, *Favolus rugulosus* Palacio & R.M. Silveira, com ocorrência no Brasil, Colômbia (Palacio *et al*. 2021, Sanchez-Ocampo *et al*. 2022), Equador e Paraguai (Veloso *et al*. 2023 Preprint), também é comestível (Palacio *et al*. 2021).

Além da presença na dieta das comunidades Sanöma, Toototobi e Waukás do povo Yanomami da Amazônia brasileira (Prance, 1972, 1973, 1984; Fidalgo & Prance, 1976; Vargas-Isla *et al*. 2013, Sanuma *et al*. 2016), basidiomas do gênero *Favolus* também são consumidos como alimento por diversas comunidades tradicionais pelo mundo. Nas Américas, registros etnomicológicos indicam o uso de basidiomas de espécies desse gênero por comunidades indígenas do México (Ruán-Solto *et al*. 2004, 2006), da Guatemala (Flores-Arzú *et al*. 2012), do Equador (Gamboa-Trujilo *et al*. 2019) e da Amazônia peruana (Vargas-Isla *et al*. 2013), assim como da Amazônia venezuelana (Zent *et al*. 2004). No continente africano, registros incluem o uso tradicional dos basidiomas como alimento em vilarejos na região de Binga, no Zimbábue (Dube *et al*. 2021), enquanto na Ásia há registros de comestibilidade em comunidades tradicionais de Malawi (Boa, 2004), no Nepal (Christensen *et al*. 2008), na Papua Nova Guiné (Boa, 2004) e nas Filipinas (De Leon *et al*. 2013).

Favolus (*Polyporaceae*, *Agaricomycetes*) é um gênero amplamente distribuído de políporos decompositores da madeira morta de diferentes espécies vegetais e cujos representantes podem ser caracterizados macromorfologicamente pelos basidiomas carnosos a coriáceos, lateralmente estipitados, subestipitados ou quase sésseis, pelo píleo espatulado, flabeliforme, reniforme ou aplanado e pela superfície do píleo radialmente estriada, glabra ou apresentando estruturas fibrilosas (Palacio *et al*. 2021). O himenóforo poroide, geralmente com poros angulares, radialmente alongados, lembrando favos de mel, mas podendo apresentar poros circulares regulares ou raramente dedaloides, é também característico (Sotome *et al*. 2013; Zhou & Cui, 2017, Palacio *et al*. 2021). Quanto à micromorfologia, o gênero é caracterizado pelo sistema hifal dimítico, com hifas esqueleto-conectivas e generativas que podem apresentar septo simples ou fibulado, pelos basidiósporos cilíndricos a naviculares, lisos e de parede fina, pelos basídios clavados, com 2 ou 4 esterigmas, e pela organização e estrutura das hifas que compõem a superfície pilear, que pode ser do tipo *rectocutis* (hifas basais ou todas

organizadas paralelamente à superfície, regulares, infladas ou não), plagiotricodérmica (hifas basais paralelas à superfície, sendo a maior parte das hifas terminais ascendendo de forma oblíqua) ou tricodérmica (hifas basais eretas, irregulares a subregulares, moderadamente infladas ou não) (Sotome *et al*. 2013; Palacio *et al*. 2021).

Em relação à ecologia, espécies de *Favolus* são decompositoras generalistas de madeira e crescem sobre troncos mortos e galhos caídos, sendo responsáveis pela podridão do tipo branca (Palacio et al. 2021). Além disso, basidiomas desse gênero são utilizadas como alimento por diferentes moscas da família Drosophilidae (Diptera) (Santa-Brígida *et al.* 2019) e estão associadas a diversos outros artrópodes, como espécies das ordens Coleoptera, Hymenoptera, Lepidoptera e das subclasses Colembola e Diplopoda (Amaringo-Cortegano *et al*. 2013).

Favolus foi originalmente descrito por Elias Magnus Fries em 1828, tendo *Favolus brasiliensis* como espécie-tipo e que foi descrita a partir de espécimes coletados na cidade do Rio de Janeiro, RJ (Fries, 1828). O gênero é atualmente aceito por vários micólogos como um gênero natural com base em estudos sistemáticos envolvendo análises morfológicas e filogenéticas moleculares (Palacio *et al*. 2021; Sotome *et al*. 2013, Zhou & Cui, 2017; Zmitrovich & Kovalenko, 2016). Apesar disso, o gênero foi tratado anteriormente, por muito tempo e por vários micólogos, como sinônimo de *Polyporus* P. Micheli ex Adans devido à sobreposição de alguns caracteres morfológicos e ecológicos, tais como: basidiomas estipitados com hábito lignícola, causadores da podridão branca; sistema hifal dimítico; e esporos lisos e cilíndricos (Corner, 1984, Ryvarden, 1991, Núñez & Ryvarden, 1995, Silveira & Wright, 2005, Drechsler-Santos, *et al.* 2008, Sotome *et al,* 2008, 2011, Dai, 2012; Gomes-Silva *et al*. 2012). Reconhecendo a heterogeneidade e diversidade macromorfológica do gênero em seu sentido amplo, *Polyporus* foi informalmente dividido em seis grandes morfogrupos infragenéricos, sendo um deles, o grupo "Favolus", caracterizado pelo píleo flabeliforme a dimidiado e o estipe lateral curto, sem uma crosta enegrecida (Núñez & Ryvarden, 1995). Sotome *et al*. (2008) fizeram o primeiro trabalho com uma abordagem filogenética molecular do gênero *Polyporus* em seu sentido amplo, utilizando sequências de nucLSU e RPB2, e evidenciaram a presença de diferentes clados bem suportados que corresponderiam a alguns dos morfogrupos propostos por Núñez & Ryvarden (1995), incluindo o grupo "Favolus". Posteriormente, Sotome *et al.* (2013) apresentaram um estudo taxonômico do grupo "Favolus" em que reconheceram, a partir de análises morfológicas e filogenéticas moleculares, utilizando sequências de ITS e nucLSU, dois gêneros, sendo eles *Favolus* e *Neofavolus* Sotome & T. Hatt., diferenciados principalmente pela organização das hifas da superfície do píleo. Zhou & Cui (2017), em seu estudo taxonômico, envolvendo análises morfológicas e filogenéticas multigênicas com oito marcadores (ITS,

nucLSU, nucSSU, mtSSU, *TBB1*, *TEF1*, *RPB1* e *RPB2*) também fizeram uma importante contribuição para com o entendimento da diversidade do gênero, com a descrição de quatro espécies novas distribuídas na Ásia e a corroboração de *Favolus* e *Neofavolus* como gêneros naturais. A última grande e importante contribuição para o estudo sistemático e filogenético de *Favolus* aconteceu com o trabalho de Palacio *et al*. (2021), que realizaram a revisão do gênero para a região Neotropical com base em análises morfológicas e filogenéticas multigênicas (ITS, mtSSU, nucLSU e *RPB1*), levando à epitipificação de *F. brasiliensis* e seu reconhecimento como um possível complexo de espécies, bem como à descrição de quatro novas espécies para a região Neotropical: *F. pseudogrammocephalus* Palacio & Drechsler-Santos, *F. radiatifibrillosus*, *F. rugulosus* e *F. yanomamii*. Atualmente, são formalmente reconhecidas cerca de 35 espécies de *Favolus* distribuídas mundialmente (Palacio *et al*. 2021), sendo que, dessas, oito ocorrem no Brasil: *F. brasiliensis* (Fries, 1828; Fidalgo, 1976; Silva-Neto *et al*. 2021; Sotome *et al*. 2013; Palacio *et al*. 2021), *F. biskeletalis* (Corner) Zmitr. & Kovalenko (Corner, 1984), *F. elongoporus* (Drechsler-Santos & Ryvarden) Zmitr. & Kovalenko (Drechsler-Santos *et al*. 2008), *F. ianthinus* (Gibertoni & Ryvarden) Zmitr. & Kovalenko (Gibertoni *et al*. 2004; Palacio *et al*. 2021), *F. pseudogrammocephalus* Palacio & Drechsler-Santos (Palacio *et al*. 2021), *F. radiatifibrillosus* (Palacio *et al*. 2021), *F. rugulosus* e *F. yanomamii* (Palacio *et al*. 2021).

Quanto ao cultivo de espécies de *Favolus,* ainda não existem espécies que são comercialmente cultivadas no mundo, e estudos de domesticação envolvendo o gênero são bastante escassos, embora alguns trabalhos tenham evidenciado sucesso no cultivo de espécies de *Favolus* em resíduos agroindustriais com formação de basidiomas para as espécies *Favolus rugulosus* (Sanchez-Ocampo *et al*. 2022, Veloso *et al*. 2023 Preprint), *Polyporus grammocephallus* Berk. [= *Favolus grammocephallus* (Berk.) Imazeki] (De Leon *et al*. 2013), e *Polyporus tenuiculus* (P. Beauv.) Fr. (= *Favolus tenuiculus* P. Beauv.) (Omarini *et al*. 2009).

Considerando o número relativamente pequeno de estudos taxonômicos incorporando análises filogenéticas e sequências de DNA de *Favolus* nos Neotrópicos, incluindo *F. brasiliensis,* este estudo, a partir de novas coletas em áreas da Mata Atlântica do Sudeste brasileiro, visa contribuir com a elucidação do complexo de espécies *F. brasiliensis,* assim como as relações filogenéticas de novas coleções de espécimes dos clados *Favolus yanomami/rugulosus* e o posicionamento filogenético de um espécime morfologicamente próximo a *F. radiatifibrillosus*. Além disso, reconhecendo o potencial para o uso dos basidiomas do gênero como alimento e a possibilidade do cultivo comercial e expansão da então restrita oferta de cogumelos comestíveis no Brasil com espécies locais, este estudo possui

também como objetivo avaliar *in vitro* o efeito de quatro temperaturas (20 °C, 25°C, 30 °C e 35°C) e quatro meios de cultura sólidos (Batata Dextrose Ágar, Malte Levedura Peptona Ágar, Levedura Glicose Ágar e Ágar Soja) e a interação desses fatores sobre o crescimento micelial em diâmetro e a produção de biomassa de isolados obtidos a partir de espécimes coletados na Mata Atlântica, sendo eles: dois isolados de *F. brasiliensis,* um isolado de *F. brunneofibrillosus* e um isolado de *F. rugulosus*.

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CAPÍTULO 1

Taxonomic reinvestigation of *Favolus* from Brazil including two new species based on morphological and multigene phylogenetic analyses

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Abstract

Favolus is a monophyletic genus of polypores with a worldwide distribution that causes white rot decomposition of various woody plant species and can be macromorphologically characterized by the fleshy and laterally stipitate basidiomata, with mostly angular and radially elongated pores. *Favolus brasiliensis,* originally described from Brazil, is the type species of the genus and has been recently suggested as a species complex based on molecular phylogenetic analyses. Morphological studies and multigene (ITS, nucLSU, *TEF1*, and *RPB1*) phylogenetic analyses of new collections of *Favolus,* including the type species *F. brasiliensis*, from the Atlantic Forest of Brazil were carried out. A total of 91 new sequences (38 ITS, 26 nucLSU, 19 *TEF1*, and 8 *RPB1*) of *Favolus* species were generated, including the first sequences of the previously described *F. radiatifibrillosus*. Despite the recognition of two supported clades of specimens identified as *F. brasiliensis* in previous phylogenies, our multigene phylogeny points towards a broader concept for the species, recovering a single broad well-supported clade for specimens identified as *F. brasiliensis*. Additionally, two new species are proposed based on morphology and multigene phylogenies: *F. brunneofibrillosus,* that is morphologically similar to *F. yanomamii* and from which it forms a sister clade, and *F. glaucovelutinus* that formed a sister clade to *F. rugulosus*. Furthermore, the inclusion of sequences of type specimens from GenBank in our phylogenetic analyses led to the elucidation of the phylogenetic position of another previously described species from Brazil, *F. elongoporus*, and to the proposal of a new combination in *Favolus* for a recently described species from Costa Rica, *Polyporus laetiporoides*. Detailed morphological descriptions of the collections and pure cultures obtained on this study are provided, along with comments, illustrations, and a key for species of *Favolus* from Brazil.

Keywords: Atlantic Forest; Brazil; Multigene phylogeny; Polyporaceae; Taxonomy; Two new taxa.

Introduction

Favolus Fr. (*Polyporaceae*, *Agaricomycetes*), with *Favolus brasiliensis* (Fr.) Fr. as the type species, is a widely distributed genus of wood-decaying polypores that causes white rot decomposition of dead wood from various plant species (Sotome et al. 2013; Zhou & Cui 2017). The genus can be macromorphologically characterized by the fleshy and laterally stipitate to substipitate basidiomata, with mostly angular and radially elongated pores (Sotome et al. 2013). Micromorphologically, the dimitic hyphal system with inamyloid skeletal-binding and generative hyphae, together with thin-walled, hyaline, smooth, and cylindrical to navicular basidiospores, are some of the diagnostic characters for the genus (Sotome et al. 2013). In addition, the pileus surface and the organization of the elements from the pileipellis has been shown to be morphological characters with a great taxonomic value for the circumscription and differentiation of the Neotropical species of *Favolus*, which can vary from a rectocutis, a plagiotrichoderm or a trichoderm (Palacio et al. 2021).

Besides its ecological importance by promoting the nutrient cycling in forest ecosystems from the decomposition of lignocellulosic substrates and its association with numerous mycophagous arthropods (Santa-Brígida et al. 2019; Falaschi et al. 2019; Amaringo-Cortegano et al. 2013), species of the genus are also economically important as wild edible mushrooms for traditional communities around the world (Boa 2004; Degreef et al. 2016; De Leon et al. 2013; Flores-Arzú et al. 2013; Gamboa-Trujilo et al. 2019; Ruán-Soto et al. 2016; Vargas-Isla et al. 2013; Zent et al. 2004). Among them, are the Sanöma, Toototobi, and Waukás communities of the Yanomami people in the Brazilian Amazon, who make use of the basidiomata of *F. brasiliensis*, *F. radiatifibrillosus* Palacio & R.M. Silveira, and *F. yanomamii* Palacio & Menolli as food (Prance, 1972, 1973, 1984; Fidalgo & Prance 1976; Vargas-Isla et al. 2013; Sanuma et al. 2016; Palacio et al. 2021).

Although the genus is currently well-accepted by mycologists as a natural group based on taxonomical studies using morphological and phylogenetic investigations (Palacio et al. 2021; Sotome et al. 2013, Zhou & Cui, 2017; Zmitrovich & Kovalenko, 2016), it has been regarded for a long time in the past as a synonym of *Polyporus* P. Micheli ex Adans due to some overlapping morphological and ecological features (Corner, 1984; Ryvarden, 1991; Núñez & Ryvarden, 1995; Kruger & Gargas 2004; Silveira & Wright 2005; Kruger et al. 2006; Drechsler-Santos et al. 2008; Sotome et al. 2008, 2011; Dai, 2012; Gomes-Silva et al. 2012) and being treated as one of the six infrageneric morphogroups of the polyphyletic genus *Polyporus* s.l., called “Favolus” (Ryvarden & Johansen, 1980; Núñez & Ryvarden, 1995; Ryvarden, 2016).

There are currently about 35 recognized species of *Favolus* distributed worldwide, although with a higher diversity in tropical regions, and with eight of them being originally described from Brazil, such as: *F. brasiliensis*, *F. biskeletalis* (Corner) Zmitr. & Kovalenko (Corner, 1984), *F. elongoporus* (Drechsler-Santos & Ryvarden) (Drechsler-Santos et al. 2008), *F. ianthinus* (Gibertoni & Ryvarden) Zmitr. & Kovalenko (Gibertoni et al. 2004), *F. pseudogrammocephalus* Palacio & Drechsler-Santos, *F. radiatifibrillosus*, *F. rugulosus* Palacio & R.M. Silveira, and *F. yanomamii* (Palacio et al. 2021).

Favolus brasiliensis has been treated previously (Nuñez & Ryvarden 1995, 2001; Silveira & Wright 2005; Ryvarden, 2016) as a synonym of *Favolus tenuiculus* P. Beauv. [=

Polyporus tenuiculus (P. Beauv.) Fr.] but the latter was then considered a dubious name because the holotype, collected in Nigeria, can't be accessed (Sotome et al. 2013; Palacio et al. 2021). Moreover, *F. brasiliensis* has recently been epitypified (Palacio et al. 2021) based on a collection from the Brazilian Atlantic Forest, the same region where the holotype was found (Fries, 1821), and it has also been interpreted as a species complex based on a molecular phylogenetic study (ITS, nucLSU and *RPB1*) that recognized two well-supported clades for samples identified under this name (Palacio et al. 2021). One of the clades, including the epitype of *F. brasiliensis*, was composed of sequences exclusively from Brazil, whilst the other was composed of sequences of specimens from Argentina, Brazil, and Costa Rica but from which the authors could not have access to the voucher collections for further taxonomical investigations (Palacio et al. 2021).

Considering the relatively small number of taxonomical studies incorporating phylogenetic analyses and DNA sequences of *Favolus* from the Neotropics, including *F. brasiliensis,* this study, based on new collections from the Atlantic Forest of Southeastern Brazil, aims to further elucidate the *F. brasiliensis* species complex, the phylogenetic relationships of new collections from the *F. yanomamii*/*rugulosus* clades, and the phylogenetic position of *F. radiatifibrillosus*, a species with a still unclear phylogenetic position.

Materials and Methods

Collections

We opted to follow the designations for Neotropical bioregions and provinces proposed by Morrone (2014) to document species distributions. Newly collections were carried out, during the most humid months (October to March) from 2020 to 2022, in areas from the Araucaria Forest, Atlantic, and Paraná Forest provinces composed of fragments of Mixed Ombrophilous, Dense Ombrophilous, and Seasonal Semideciduous Forest types (Oliveira-Filho & Fontes, 2000; Veloso et al. 1992) in Brazil. Collection sites include the following protected areas: '*Parque Estadual da Cantareira*' (PEC); '*Parque Estadual das Fontes do Ipiranga*' (PEFI); '*Parque Estadual da Serra do Mar*' (PESM); '*Parque Estadual Turístico do Alto Ribeira*' (PETAR), and '*Parque Nacional da Serra da Bocaina*' (PNSB). The collections studied are deposited at the Fungarium IFungiLab (FIFUNGI), FLOR and SP (Thiers, 2023, 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 #A818A15).

Morphological studies

Macromorphological characters were described mostly from color photographs of fresh specimens collected in the field and from dried specimens. Fresh collections and cultures were photographed with a Canon Rebel T3i or Canon 6D Mark II camera (Canon Corporation, Tokyo, Japan). Measurements of macromorphological characters are based on fresh specimens, unless specified. Basidiomata were dried in a food dehydrator at 45 ºC and dehydrated vouchers were stored with allochroic silica gel for their preservation. Basidiomata and culture colors were described following Kornerup &Wanscher (1978). Odor and taste were not recorded.

Microscopic analyses were performed with Zeiss Axioscope 5 microscope (Carl Zeiss AG, Oberkochen, Germany) on bright field using freehand sections of dried materials that were rehydrated in 5% KOH or 3% NaOH and mounted in 5% KOH and/or 1% Congo Red or 1% phloxine. Cyanophilous and amyloid reactions were observed in Cotton Blue and Melzer's reagent, respectively. Micromorphological characters were photographed using Zeiss Axiocam 208 Color at magnifications up to 1000× and measured with Zeiss Zen 3.1 Blue edition software.

The following abbreviations were used: IKI− = inamyloid and nondextrinoid; CB+/− = cyanophilous/acyanophilous; m = arithmetic mean; and Q = the ratio of length/width of basidiospores. To better determine the size range of basidiospores, 5% of the measurements at each end of the range are noted in parentheses. At least 30 basidiospores of each collection were measured. Basidiospores size measurements are annotated as n = number of basidiospores measured/number of basidiomata per collection analyzed/number of collections.

The form of the basidiospores was interpreted based on the Q values following Bas (1969). Micromorphological characters of the basidiomata were described based on Clémençon (2004).

Cultural studies

Pure cultures were isolated from tissue fragments obtained from the context of the pileus or stipe of fresh basidiomata that were then cultured and maintained in sterile Petri plates containing Potato Dextrose Agar (PDA, Kasvi, Spain) and incubated at 25 ºC, with trimestral subculturings. The identity of the pure cultures was confirmed based on phylogenetic analyses of sequences from the nuclear rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) obtained from DNA extracted. Macromorphological characters, such as the mycelium form at the marginal zone, the texture of the aerial mycelium, formation of crusts and exudates, the colony color, and color alterations of the medium beneath the mycelium, as well as micromorphological characters, such as the distribution of clamps, hyphal width, hyphae differentiations and asexual reproductive structures, from the cultures grown in PDA and maintained at 25 °C were described using Stalpers (1978). Pure cultures were preserved in sterile distilled water (Castellani, 1967) and are deposited at the 'Coleção de Culturas de Algas, Cianobactérias e Fungos' (CCIBt) of the Instituto de Pesquisas Ambientais, São Paulo, Brazil.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dried specimens or pure cultures using the Doyle and Doyle (1987) protocol adapted by Góes-Neto *et al*. (2005) or with the DNeasy Plant Mini Kit (Qiagen, Cat. # 69104, Valencia, CA, USA), following the product instructions.

The ITS, the partial regions of nuc28S rDNA (nucLSU), the translation elongation factor 1 alpha (*TEF1*), and the largest subunit of RNA polymerase II (*RPB1*) were amplified by polymerase chain reactions (PCR). Four primer pairs were used: ITS1-F/ITS4 (White *et al*. 1990) for ITS, LR0R/LR5 (Vilgalys & Hester, 1990; James *et al.* 2006) for nucLSU, 983F/1567R (Rehner & Buckley, 2005) for *TEF1*, and gRPB1-Af/fRPB1-Cr (Matheny *et al*. 2002) for *RPB1*.

The PCR reactions were conducted on a Mastercycler Nexus GX2 thermal cycler (Eppendorf SE, Cat. # 6336000023, Hamburg, Germany). The amplification reaction mixture contained 25 µl Taq Pol - Master mix (2×) Green (Cellco Biotech, Cat. # POL-102XL, São Paulo, Brazil), 2.5 µL of each primer, 15 µL of ultrapure water, and 5 µL of template DNA. Thermal profile of PCR for DNA markers was according to Binder & Hibbett (2003) and Palacio *et al*. (2021). After visualization of positive PCR products on a 2% agarose gel with Safedye Nucleic Acid Stain (Cellco Biotech, Cat. # PCK-301S), PCR products were cleaned up prior to sequencing using a QIAquick PCR Purification Kit (Qiagen, Cat. # 28106) or PCR Purification Kit DPK-106 (Cellco Biotech, Cat. # DPK-106L) as per manufacturer's guidelines. Sanger sequencing was carried out at the 'Centro de Estudos do Genoma Humano e Células-Tronco – USP' (São Paulo, Brazil) or at Macrogen Korea (Seoul, South Korea). Sequencing was carried out using the same primers as those used for PCR. Raw data were processed using Geneious Prime v2023.1.1 (Dotmatics, Bishop's Stortford, UK). New sequences are deposited at GenBank and the accession codes are indicated at Table 1.

Molecular and Phylogenetic analyses

A combined dataset of ITS, nucLSU, *TEF1*, and *RPB1* of the newly generated sequences and related sequences available in GenBank was assembled (Table 1) based on Palacio *et al*. (2021) and Zhou & Cui (2017). Sequences were aligned in MAFFT 7 (Katoh & Standley, 2013) under the E-INS-I strategy for ITS and G-INS-I strategy for nucLSU, *TEF1*, and *RPB1*. Alignments were manually adjusted with Aliview 1.17.1 (Larsson 2014) to minimize ambiguously aligned sites. Individual gene alignments were concatenated using Mesquite v.3.81 (Maddison & Maddison 2023). The IQ-TREE2 software (Minh et al. 2020) was used for the selection of the best partitioning scheme and the best-fit substitution model for each partition with ModelFinder (Kalyaanamoorthy et al. 2017) and forcing the software to search for substation models supported by MrBayes. The final alignment is deposited at TreeBASE (31073). The evaluation of the pairwise divergence of ITS sequences and the summarization of the number of invariable, variable, and informative sites was performed on PAUP 4.0 (Swofford, 2002).

Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed with the combined dataset in the CIPRES Scientific Gateway (https://www.phylo.org/portal2/home.action, Miller et al. 2010). The BI analysis was performed using the defined partitions and evolutionary models in MrBayes 3.2 (Ronquist et al. 2012) with four independent runs, each one with four chains and starting from random trees. The runs performed 10 million generations and trees were sampled every 1000th generation. Twenty-five percent of the sampled trees were discarded as burn-in, while the remaining ones were used for calculating the consensus tree and Bayesian Posterior Probabilities (BPP). 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. 2014). The ML analysis for phylogeny and assessment of branch support by Bootstrap Support (BS) percentages was carried out using the RAxML-HPC v.8 on XSEDE (8.2.12) (Stamatakis 2014). The analysis first involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree, under a GTRGAMMA model, with all the other parameters estimated by the software. We uploaded the partition file with the defined partitions to force RAxML to search for a separate evolution

model for each partition. The BS values were obtained under the same models and partitioning schemes allowing the program to automatically halt bootstrapping using the autoMRE option. A node was considered strongly supported if it showed BPP ≥ 0.95 and/or BS ≥ 80%. Sequences of *Trametes conchifer* (Schwein.) Pilát were used as the outgroup based on Zhou & Cui (2017).

Table 1: List of species, specimen vouchers, geographic origins, and GenBank accession numbers for the sequences used in the molecular phylogenetic analyses of this study.

Species	Specimen no.	Country	Accession number			
			ITS	nucLSU	*TEF1*	*RPB1*
Cerioporus squamosus	Cui10595*	China	KU189778	KU189809	KU189925	KU189892
Datronia mollis	RLG6304sp	USA	JN165002	JN164791	JN164901	JN164818
Favolus acervatus	Cui11053*	China	KU189774	KU189805	KU189920	KU189889
Favolus acervatus	Dai10749b	China	KX548953	KX548979	KX549043	KX549065
Favolus brasiliensis	DAZ003	Brazil	**OR353428**	**OR351889**	—	—
Favolus brasiliensis	DAZ014	Brazil	**OR353429**	**OR351890**	—	—
Favolus brasiliensis	DAZ016	Brazil	**OR353430**	—	**OR735301**	—
Favolus brasiliensis	DAZ017	Brazil	**OR353431**	—	—	—
Favolus brasiliensis	DAZ025	Brazil	**OR353432**	**OR351891**	—	—
Favolus brasiliensis	DAZ066	Brazil	**OR353433**	—	—	—
Favolus brasiliensis	DAZ070	Brazil	**OR353434**	—	**OR735302**	—
Favolus brasiliensis	DAZ071	Brazil	**OR353435**	**OR351892**	**OR735303**	**OR735320**
Favolus brasiliensis	DAZ074	Brazil	**OR353436**	**OR351893**	**OR735304**	**OR735321**
Favolus brasiliensis	DAZ083	Brazil	**OR353437**	—	**OR735305**	—
Favolus brasiliensis	DAZ086	Brazil	**OR353438**	**OR351894**	**OR735306**	**OR735322**
Favolus brasiliensis	DAZ090	Brazil	**OR353439**	**OR351895**	**OR735307**	—
Favolus brasiliensis	DAZ101	Brazil	**OR353440**	**OR351896**	**OR735308**	**OR735323**
Favolus brasiliensis	DAZ102	Brazil	**OR353441**	**OR351897**	**OR735309**	**OR735324**
Favolus brasiliensis	DAZ103	Brazil	**OR353442**	—	—	—
Favolus brasiliensis	DAZ104	Brazil	**OR353443**	**OR351898**	—	—
Favolus brasiliensis	FLAS-F-61023	USA	MH211695	—	—	—
Favolus brasiliensis	OMC1466	USA	KY948796	—	—	—
Favolus brasiliensis	HONDURAS19-F099*	Honduras	MT571537	—	—	—
Favolus brasiliensis	INPA241452	Brazil	AB735977	AB735953	—	—

Species	Specimen no.	Country	Accession number			
			ITS	nucLSU	*TEF1*	*RPB1*
Favolus brasiliensis	Kellermann s.n	Brazil	MN648682	MN648708	—	—
Favolus brasiliensis	MCW576	Brazil	—	MN648710	—	—
Favolus brasiliensis	MEEsnbras	Brazil	—	MN648711	—	—
Favolus brasiliensis	MO310538	Mexico	MH158253	—	—	—
Favolus brasiliensis	MP197	Brazil	MN648683	MN648709	—	MN781127
Favolus brasiliensis	MPCS049	Brazil	**OR502610**	**OR351899**	—	—
Favolus brasiliensis	NMJ343	Brazil	**OR353444**	**OR351900**	**OR735311**	—
Favolus brasiliensis	NMJ348	Brazil	**OR353445**	**OR351901**	**OR735312**	—
Favolus brasiliensis	NMJ409	Brazil	**OR353446**	**OR351902**	**OR735313**	**OR735326**
Favolus brasiliensis	TENN10242*	Costa Rica	AB735976	AB368097	—	—
Favolus brasiliensis	TENN11415	Argentina	—	AB368098	—	—
Favolus brasiliensis	MPD708*	Brazil	**OR346135**	**OR351910**	**OR735310**	**OR735325**
Favolus brasiliensis	MPD715*	Brazil	**OR346136**	—	—	—
Favolus brunneofibrillosus	DAZ009	Brazil	**OR353453**	**OR351906**	**OR735317**	—
Favolus brunneofibrillosus	DAZ139	Brazil	**OR353454**	**OR351907**	**OR735318**	—
Favolus brunneofibrillosus	MPD711*	Brazil	**OR346137**	**OR351908**	—	—
Favolus brunneofibrillosus	NMJ441	Brazil	**OR353455**	**OR351909**	**OR735319**	—
Favolus elongoporus	O-F-450234	Brazil	MT216227	—	—	—
Favolus emerici	Yuan4410	China	KX548954	KX548980	KX549044	KX549066
Favolus emerici	Cui10926*	China	KU189776	KU189807	KU189922	KU189890
Favolus glaucovelutinus	DAZ207	Brazil	**OR353452**	**OR351905**	**OR735316**	—
Favolus glaucovelutinus	DAZ270	Brazil	**PP062816**	**PP062815**	—	—
Favolus gracilisporus	BP106942	Hungary	MF401551	—	—	—
Favolus gracilisporus	SFC2013070440	South Korea	KY038472	—	—	—
Favolus ianthinus	DS1677	Brazil	MN648691	MN648720	—	—
Favolus ianthinus	DS1700	Brazil	MN648690	MN648719	—	—
Favolus laetiporoides	JV1704-13-2	Costa Rica	MN272352	—	—	—
Favolus niveus	Cui11129	China	KX548955	KX548981	KX549045	KX549067
Favolus niveus	Dai13276	China	KX548956	KX548982	KX549046	KX549068
Favolus pseudobetulinus	TRTC51022	Canada	AB587629	AB587620	—	—

Species	Specimen no.	Country	Accession number			
			ITS	nucLSU	*TEF1*	*RPB1*
Favolus pseudobetulinus	TFMF27567	Japan	AB587644	AB587639	—	—
Favolus pseudoemerici	Cui11079	China	KX548958	KX548984	KX549048	KX549069
Favolus pseudoemerici	Cui13757T	China	KX548959	KX548985	KX549049	—
Favolus pseudogrammocephalus	MP218	Brazil	MN648689	MN648717	—	MN781128
Favolus pseudogrammocephalus	MP220	Brazil	—	MN648718	MT895849	MN781129
Favolus radiatifibrillosus	FLOR68429	Brazil	**PP062817**	—	—	—
Favolus radiatifibrillosus	MPD579	Brazil	**OR353447**	**OR351903**	**OR735314**	—
Favolus radiatifibrillosus	SP-Fungi 49734	Brazil	**PP062818**	—	—	—
Favolus roseus	PEN33	Malaysia	AB735975	AB368099	—	—
Favolus roseus	GVM-787	India	MT012371	MT012370	—	—
Favolus rugulosus	MP191	Brazil	MN648684	MN648712	—	MN781130
Favolus rugulosus	Fazolino415	Brazil	MN648685	MN648713	—	—
Favolus rugulosus	DAZ079	Brazil	**OR353448**	**OR351904**	**OR735315**	**OR735327**
Favolus rugulosus	DAZ189a	Brazil	**OR353449**	—	—	—
Favolus rugulosus	DAZ215	Brazil	**OR353450**	—	—	—
Favolus rugulosus	HUA228632	Colombia	ON897745	—	—	—
Favolus rugulosus	MAC011	Brazil	**OR502609**	**OR502617**	—	—
Favolus rugulosus	MP191	Brazil	MN648684	MN648712	—	MN781130
Favolus rugulosus	MPD240*	Brazil	**OR346134**	—	—	—
Favolus rugulosus	NMJ376	Brazil	**OR353451**	—	—	—
Favolus rugulosus	NMJ459	Brazil	**OR502611**	**OR502618**	—	—
Favolus rugulosus	NMJ460	Brazil	**OR502612**	**OR502619**	—	—
Favolus rugulosus	RLC1259*	Ecuador	OQ871942	OQ913011	—	—
Favolus septatus	Zhou287	China	KX548968	—	KX549054	—
Favolus sp.	MEL2382969	Australia	KP012829	KP012829	—	—
Favolus spatulatus	Dai13615*	China	KU189775	KU189806	KU189921	—
Favolus spatulatus	Cui8290	China	KX548969	KX548991	KX549055	—
Favolus subtropicus	Cui4292	China	KX548970	KX548992	KX549056	—
Favolus subtropicus	Li1938	China	KX548971	KX548993	KX549057	KX549070
Favolus yanomamii	ACM1295	Brazil	MN648686	MN648714	—	—
Favolus yanomamii	DS1656	Brazil	MN648687	MN648715	—	—

Species	Specimen no.	Country	Accession number			
			ITS	nucLSU	*TEF1*	*RPB1*
Favolus yanomamii	MEEsnNbras	Brazil	MN648688	MN648716	—	MN781131
Lentinus badius	DED07668	Thailand	KP283480	KP283518	—	KP325692
Lentinus tigrinus	LE214778	Russia	KM411459	KM411475	KM411490	—
Neofavolus alveolaris	Dai11290*	China	KU189768	KU189799	KU189913	KU189885
Neofavolus mikawae	Dai12361	China	KX548975	KX548997	KX549061	—
Neofavolus teixeirae	AL60	Brazil	MG675058	MG675059	—	—
Polyporellus arcularius	Cui10998	China	KX548973	KX548995	KX549059	KX549071
Polyporellus brumalis	Cui10750*	China	KU189765	KU189796	KU189910	KU189883
Trametes conchifer	FP106793sp	USA	JN164924	JN164797	JN164887	JN164823

Notes: New sequences generated in this study are marked in bold. Specimen vouchers followed by an asterisk indicates sequences generated from pure cultures.

Results

Molecular and Phylogenetic Analyses

A total of 91 new sequences of five species of *Favolus* were generated. Of these, 38 sequences are from the ITS region, 26 from nucLSU, 19 from *TEF1*, and eight from *RPB1*. The concatenated alignment consisted of 3,641 characters including 2,133 constant characters, 340 variable characters that are not informative and 1168 phylogenetically informative sites. The best partitioning scheme was seven-subset: 1) ITS1 and ITS2; 2) 5.8S, *TEF1* 1st and 2nd codon positions of the first coding region, *TEF1* 3nd codon position of the third coding region, *RPB1* 1st codon position of the first coding; 3) 28S, *TEF1* 1st and 2nd codon positions of the second coding region, *TEF1* 2nd codon position of the third coding region, *RPB1* 2nd codon position of the first coding region, *RPB1* 2nd and 3rd codon positions of the second coding region and *RPB1* 1st and 3rd codon positions of the third coding regions; 4) *TEF1* 3rd codon position of the first coding region, *TEF1* 3rd codon position of the second coding region and *TEF1* 1st codon position of the third coding region; 5) *TEF1* introns; 6) *RPB1* 1st codon position of the second coding region, *RPB1* 2nd codon position of the third coding region and *RPB1* first, third and fourth introns; 7) *RPB1* 3rd codon position of the first coding region and *RPB1* second intron. The best-fit substitution models for each partition were: HKY+F+G4 for subset 1, K2P+FQ+I for subset 2, GTR+F+I+R2 for subset 3, HKY+F+G4 for subset 4, HKY+F+I+R2 for subset 5, HKY+F+I+G4 for subset 6 and GTR+F+G4 for subset 7. The topology of the BI and ML analyses showed no major inconsistencies in most clades, differing only in the *F. brasiliensis* clade where in the Maximum Likelihood reconstruction, samples were recovered in various weakly supported subclades (< 50 BS). Due to a better topology of the *F. brasiliensis* clade in the multigene Bayesian phylogeny, the BI topology is presented in Fig. 1. *Favolus* (1 BPP/100 BS), *Lentinus*/*Polyporellus* (1 BPP/100 BS), and *Neofavolus* (1 BPP/100 BS) are recovered as three monophyletic well-supported clades. Two major clades within *Favolus* are

recovered with strong support in accordance with Palacio et al. (2021): one represented by sequences exclusively from the USA, Mexico, and Central and South America (mostly from Brazil) that corresponds to five species, and the other composed of at least thirteen species from sequences mostly from Asia but also including sequences from Canada, Hungary, and Brazil, with the latter country represented by five species. Examined specimens in this study were placed into four main clades:

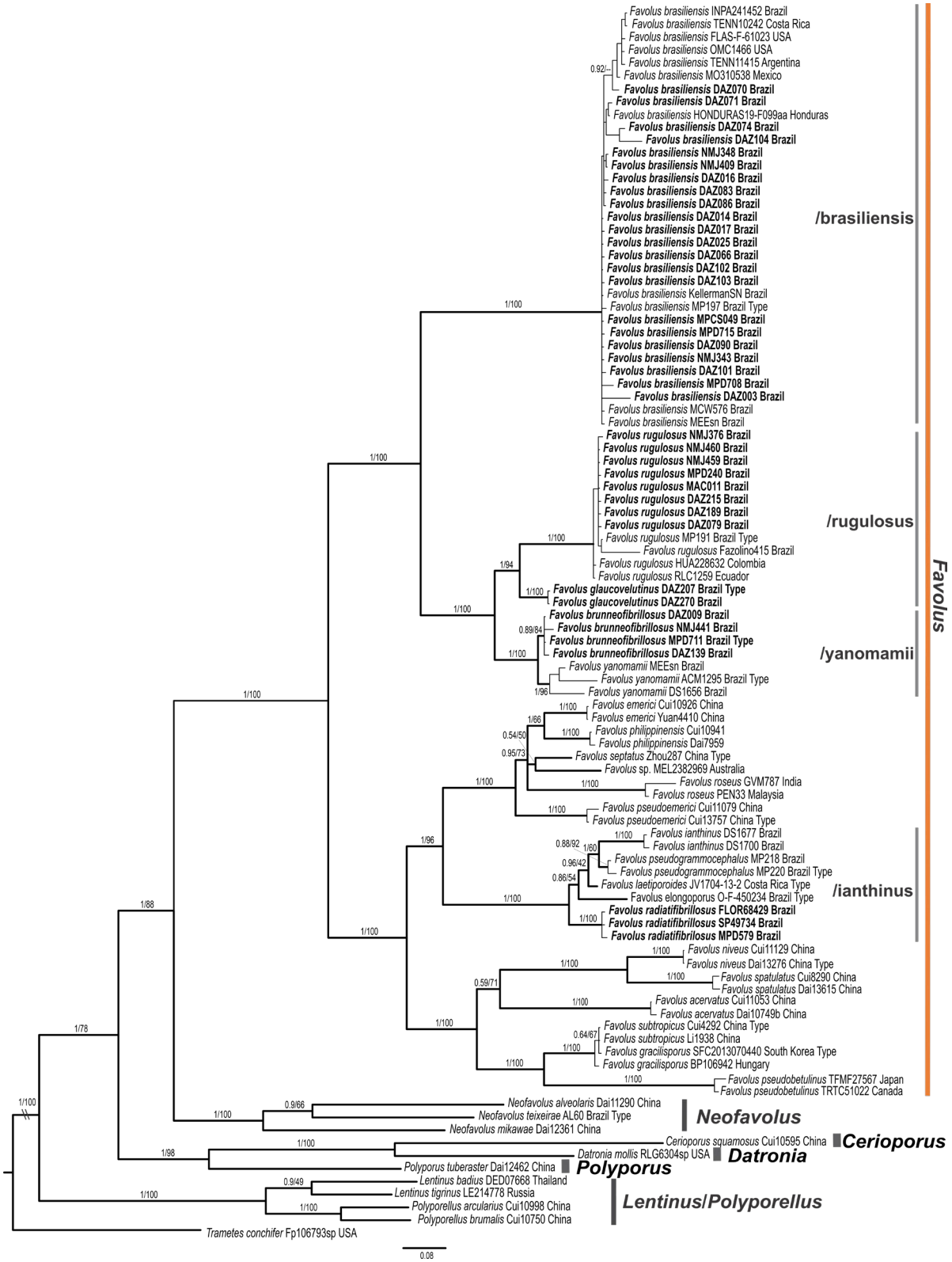

Fig. 1. Bayesian Inference phylogeny of the combined (ITS+nucLSU+*TEF1*+*RPB1*) dataset. The BPP and BS values are provided above the branches. Sequences from samples generated in this study are indicated in bold.

i) /brasiliensis clade (1 BPP/100 BS) that is represented by 31 specimens of *F. brasiliensis*, most of them from Brazil but also including sequences from Argentina, Costa Rica, Honduras, Mexico, and two sequences from Florida state in the USA. Pairwise divergence of ITS sequences of *F. brasiliensis* ranged from 0.08% to 3.04%.

ii) /rugulosus clade (1 BPP/94 BS) that is represented by *F. glaucovelutinus* from Brazil and *F. rugulosus* from Brazil, Colombia, and Ecuador.

iii) /yanomamii clade (1 BPP/100 BS) that is represented by *F. yanomamii* and *F. brunneofibrillosus*, both from Brazil. Pairwise divergence of ITS sequences between *F. yanomamii* and *F. brunneofibrillosus* varies from 1.0 % to 3.6 %, whilst the sequences of *F. yanomamii* are 97.28–99.49% identical and the sequences of *F. brun0neofibrillosus* are 99.67–100% identical.

iv) /ianthinus clade (1 BPP/100 BS) that is represented by *F. ianthinus, F. pseudogrammocephalus*, *F. elongoporus*, *F. radiatifibrillosus,* all from Brazil, and *F. laetiporoides* from Costa Rica.

Phylogenetic and morphological analyses revealed three new lineages with distinct morphological and molecular characters that separate them from other known species of *Favolus*, one corresponding to the previously described *F. radiatibrillosus* and the two other that are proposed here as new species, viz. *F. brunneofibrillosus* and *F. glaucovelutinus*. Additionally, sequences of type specimens of the previously described species included in our phylogenetic analyses, *F. elongoporus* and *Polyporus laetiporoides* Vlasák & Ryvarden were recovered in *Favolus.* Also, despite sequences of ITS and/or nucLSU identified as *F. brasiliensis* from Argentina, Brazil, Costa Rica, Honduras, and the USA formed a supported internal subclade (0.92 BPP/-BS) in our Bayesian reconstructed phylogeny, all specimens identified as *F. brasiliensis* are recovered in a single well-supported main clade (1 BPP/100 BS), and thus we interpret this phylogenetic divergence as a case of intraspecific variability (see discussion under *F. brasiliensis* taxonomy). Description of the new taxa and other studied species of *Favolus* from Southeastern Brazil along with comments for each species are presented on the following Taxonomy section.

Taxonomy

Favolus brasiliensis (Fr.) Fr., Linnaea 5:511 (1830) Figs. 2, 3, 8d, 13a, d

MycoBank MBT395310

≡ *Daedalea brasiliensis* Fr., Syst Mycol 1:332 (1821)

= *Favolus fissus* Lév., Ann Sci Nat Bot 2:201 (1844)

= *Favolus alutaceus* Berk. & Mont., Ann Sci Nat Bot 11:240 (1849)

= *Favolus giganteus* Mont., Ann Sci Nat Bot 1:135 (1854)

= *Favolus hispidulus* Berk. & M.A. Curtis, J Linn Soc Bot 10:321 (1869)

= *Favolus paraguayensis* Speg., Anal Soc Cient Argent 17(2):71 (1884)

= *Favolus speciosus* Speg., Anal Soc Cient Argent 17(2):71 (1884)

= *Favolus fimbriatus* Speg., Anal Soc Cient Argent 17(2):72 (1884)

= *Favolus saltensis* Speg., Anal Mus Nac Hist Nat Buenos Aires 6:176 (1898)

= *Favolus pseudoprinceps* (Murrill) Sacc. & Trotter, Syll. fung. (Abellini) 21: 355 (1912)

= *Favolus bresadolianus* Speg., Boln Soc Cienc Córdoba 28:353 (1926)

= *Favolus brasiliensis* var. *fimbriatus* (Speg.) Rick, Iheringia 7:264 (1960)

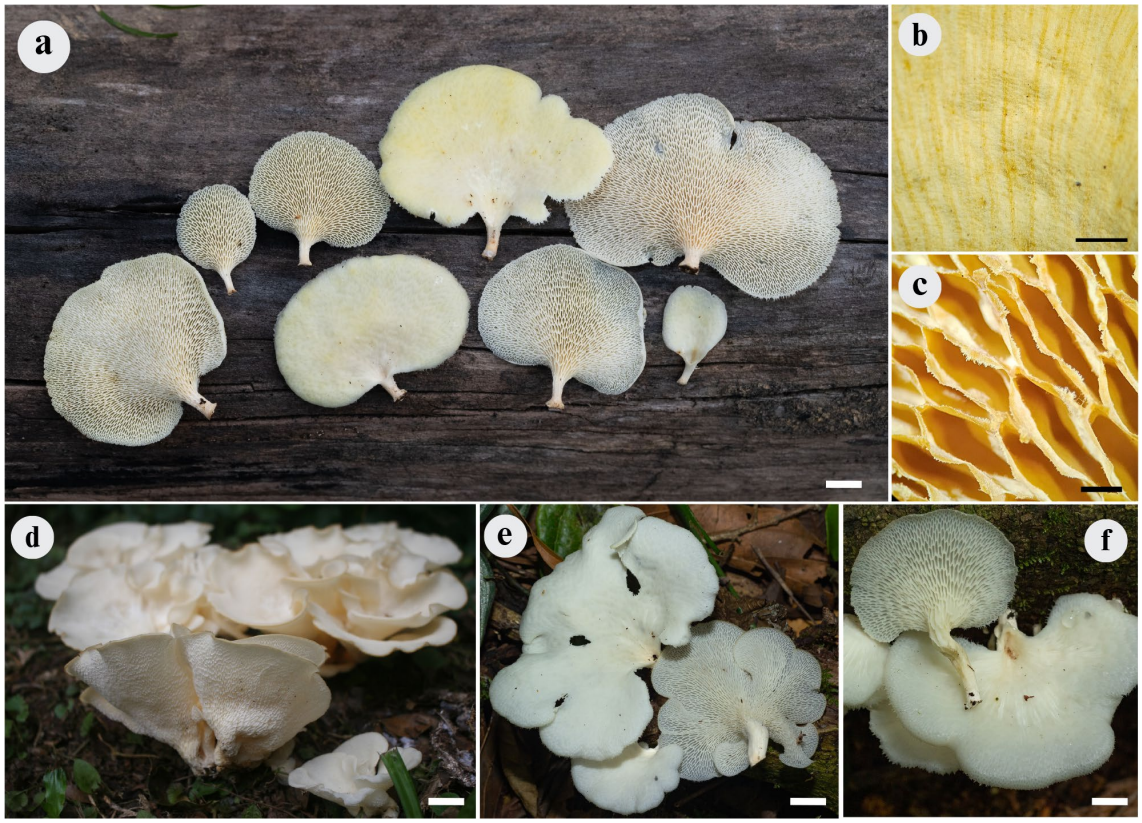

Fig. 2 *Favolus brasiliensis.* a, d–f. Basidiomata. b. Details of the pileus surface. C. Details of the pores. a–c. DAZ90. d. MPD465. e. DAZ70. f. DAZ181. Black scale bars = 0,5 cm and white scale bars = 1 cm. Photos: a–c, e, f. D.A. Zabin; d: Mariana P. Drewinski.

Basidiomata annual, substipitate to laterally or eccentrically to centrally stipitate, gregarious, clustered to caespitose. Pileus 15–100 mm wide, 14–90 mm from the base to the margin, 1–3 mm thick; circular, spathulate, reniform to flabelliform, plane to laterally infundibuliform; surface glabrous towards the margin to irregularly pubescent or hispid closer to the stipe, radially striate with whitish to yellowish lines; pure white to pale yellow (2A2), becoming light yellow (1A5) with age; margin entire, acute, sometimes becoming yellowish brown to brown (5B6 to 6E8) when dry, and rarely somewhat ciliate or fimbriate; context 1 mm

thick, pale white (2A2). Pore surface cream to pale orange (5A3), pores 1–10 mm × 0.5–1.8 mm, angular, radially elongated to rather stretched lengthwise, decurrent; dissepiments entire or lacerate to sometimes fringed; tubes 1 mm long, concolorous with the pore surface. Stipe 5–16 × 2–7 mm; subcylindrical to cylindrical, solid; glabrous to pubescent or hispid towards the base; concolorous with the pileus surface.

Hyphal system dimitic with generative and skeletal-binding hyphae. Generative hyphae 2.0–4.8 µm wide, with clamp connections or simple septa, hyaline, thin-walled, rare, more frequent in younger basidiomata. Skeletal-binding hyphae 2.0–8.5 µm wide, thick-walled, flexuous, branched, hyaline, nondextrinoid, abundant. Hyphal pegs 44–82 × 16.0–36 µm, frequent, composed of generative hyphae. Pileipellis a rectocutis, 6.0–20 µm thick, composed of generative hyphae that are repent, clamped, and hyaline, 1.5–3.0 µm wide. Basidia 16–32 × 5.0–7.6 µm, clavate, 2- or 4-sterigmate; basidioles 14.0–28 × 4.0–6.4 µm, clavate. Basidiospores (7.0–)7.8–10.8(–11.6) × (2.5–)2.9–3.8(–4.4) µm (m = 9.2 × 3.3 µm), Q = 2.0–3.8 (m = 2.77; n = 300/1/15), cylindrical or bacilloid, thin-walled, smooth, hyaline, guttulate, frequently with one or two large guttules, IKI–, CB–.

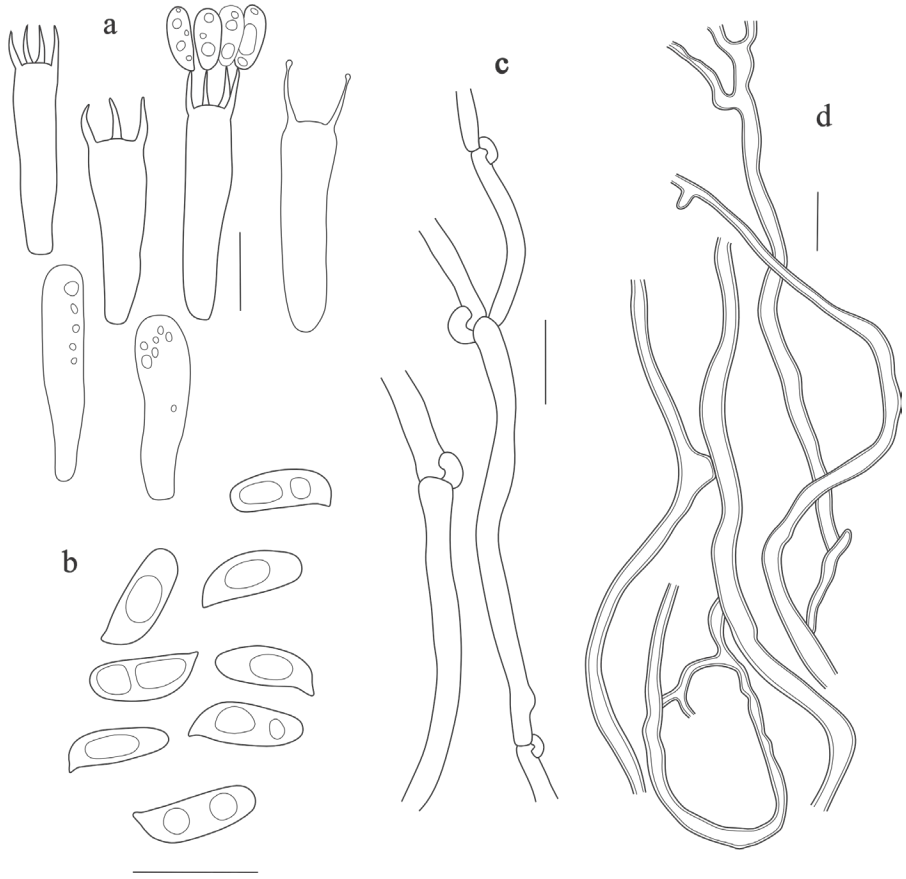

Fig. 3 Microcharacters of *F. brasiliensis* (DAZ90). A. Basidia and basidioles. B. Basidiospores. C. Generative hyphae. D. Skeletal-binding hyphae. Scale bars = 10 µm. Line drawings by C.C. Nascimento.

Culture: Mycelium growing prostrate to the medium but sometimes also submerged at the margin. Aerial mycelium mostly growing very appressed, thin, felty to subfelty with thicker hyphal bundles, sometimes growing in a reticulate manner with some cottony mycelium tufts

arising from the surface, or else cottony and rarely coarse and granular; with fimbriate margin; mycelium mat white to yellowish white, with pale yellowish or brownish orange patches, mostly from the center of the culture, sometimes with yellowish exudates droplets forming on the surface. Basidiomata primordia growing on the border wall of older cultures. Reverse unaltered. Generative hyphae with frequent clamp connections, thin-walled, hyaline, 2.5–5.0 µm wide, more abundant on the marginal zone; skeletal hyphae 3.0–4.5 µm wide, thick-walled, flexuous, branched, sometimes growing in a serpent-like manner through the mycelium, more frequently in the inner zone of the culture. Arthroconidia present more frequently in the marginal zone, composed of fragmented hyphae of variable length, which can also be rather ellipsoid with thickened walls.

Ecology and distribution: On wood of *Cecropia* sp., *Couma macrocarpa* Rodr., *Croton palanostigma* Klotzsch, *Dioclea* cf. *malacocarpa* Ducke, *Ficus* sp., *Inga edulis* Mart., *Micropholis melinoniana* Pierre, *Inga edulis* Mart., *Micropholis* sp., *Pourouma* sp., *Sagotia racemose* Baill., *Sterculia* sp. (Sanuma et al. 2016), and other unidentified angiosperms. Known from the Cuban province in the Antillean subregion (Palacio et al. 2021). Brazilian subregion in Guianan Lowlands, Imeró, Magdalena, Pacific Lowlands, Pantepui (Palacio et al. 2021) and Mosquito provinces; Chacoan subregion in Araucaria Forest, Atlantic, Chacoan, Cerrado, Pampean, Parana Forest, and Xingu-Tapajós provinces (Palacio et al. 2021). Pacific subregion in Costa Rica and in the Austroriparian province from the Alleghany subregion of the Nearctic region (Escalante et al. 2021). January to July, September to December.

Specimens examined: BRAZIL: PARANÁ: Guarapuava, Rural Area, 1 March 2023, *M.P. Drewinski MPD715* (FIFUNGI-272); RIO DE JANEIRO: Paraty, Estrada Real Trail, 23°12'10.0"S 44°47'53.7"W, 9 February 2021, *D.A. Zabin DAZ03* (FIFUNGI-106); SÃO PAULO: Caraguatatuba, Parque Estadual da Serra do Mar, Núcleo Caraguatatuba, Jequitibá Trail, 23°35'38.3"S 45°25'37.8"W, 25 January 2022, *D.A. Zabin DAZ66* (FIFUNGI-131); ibid., *DAZ70* (FIFUNGI-133); *ibid.*, *DAZ71* (FIFUNGI-134); *ibid.*, *DAZ74* (FIFUNGI-143); Eldorado, 4 November 2022, *N. Menolli Jr. NMJ461* (FIFUNGI-208); *ibid.*, *NMJ466* (FIFUNGI-211); Iporanga, Reserva Betary, 24°35'19.1"S 48°37'40.7"W, 4 March 2021, *D.A. Zabin DAZ14* (FIFUNGI-109); *ibid.*, *DAZ17* (FIFUNGI-111); Juquitiba, Instituto Terra Luminous, Chama Violeta Trail, 24°01'17.7"S 47°03'58.6"W, 9 February 2022, *D.A. Zabin DAZ83* (FIFUNGI-178); *ibid.*, *DAZ86* (FIFUNGI-180); *ibid*. Jaguatirica Trail, 24°01'17.3"S 47°04'11.5"W, 9 February 2022; ibid., *D.A. Zabin DAZ90* (FIFUNGI-181); *ibid*., Head office, 2 October 2021, *N. Menolli Jr. NMJ409* (FIFUNGI-174); Monte Alegre do Sul, Fazenda da Agência Paulista de Tecnologia dos Agronegócios, Water tank trail, 6 March 2020, *N. Menolli Jr. NMJ348* (FIFUNGI-106); São Paulo, Parque Estadual da Cantareira, Núcleo Pedra Grande, 23°27'09.5"S 46°38'18.9"W, 22 November 2021, *D.A. Zabin DAZ25* (FIFUNGI-113); *ibid.*, 26 February 2021, *M.P.C. Santos MPCS49* (FIFUNGI-173); São Paulo, Parque Estadual das Fontes do Ipiranga, close to the second entrance gate, 23°38'40.6"S 46°37'27.3"W, 28 February 2020, *N. Menolli Jr. NMJ343* (FIFUNGI-166); Ubatuba, Parque Estadual da Serra do Mar, Núcleo Picinguaba, Road to Camburi beach, 23°21'39.6"S 44°46'12.4"W, 16 February 2022, *D.A. Zabin DAZ101* (FIFUNGI-185); *ibid*., *DAZ102* (FIFUNGI-186); *ibid.*, *DAZ103* (FIFUNGI-187); *ibid*., *DAZ104* (FIFUNGI-188).

Cultures examined: CCIBt 4768, obtained from collection *MPD708* (Brazil, São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 23°38'40.6"S 46°37'27.3"W) and isolated by M.P. Drewinski on 21 February 2022; CCIBt 4770, from collection *MPD715* (Brazil, Paraná: Guarapuava, Rural Area) and isolated by M.P. Drewinski on 1 March 2023.

Comments: *Favolus brasiliensis* is easily distinguished from the other Neotropical species of the genus with angular and radially elongated pores by the whitish and glabrous pilear surface with radial yellowish to whitish lines, the pores that are generally stretched lengthwise with short tubes, and microscopically by the pileipellis as a rectocutis and the generative hyphae with clamp connections. Despite this, *F. brasiliensis* can be rather variable in relation to its macromorphology which could be responsible to the large number of synonyms proposed by examination of type specimens described from the Neotropical region, as also accepted here (Palacio et al. 2021). Based on the original description of *Favolus pseudoprinceps* (Murrill) Sacc. & Trotter [as *Hexagonia pseudoprinceps* Murrill] described from Puerto Rico (Murrill, 1907), which is known only from the type specimen, we recognize it as another synonym of *F. brasiliensis* because they share a pileus that can be reniform with a glabrous and a delicate radially lined surface, long pores (4–6 mm) that are radially elongated and decurrent, and a lateral or eccentric stipe that is glabrous or sometimes hispid (Murrill, 1907). Micromorphological characters from our pure cultures of *F. brasiliensis* agrees with previous descriptions, such as the presence of generative hyphae with clamp connections, branched thick-walled skeletal hyphae, and the presence of arthroconidia (Motato-Vásquez et al. 2016; Neves, 1998, both as *F. tenuiculus* but that most likely represent *F. brasiliensis*), but differs by the apparent lack of crystals, which were present on the cultures studied by Neves (1998 as *F. tenuiculus*). *Favolus brasiliensis* was previously recognized as a species complex based on multigene (ITS, nucLSU, mtSSU, and *RPB1*) phylogenetic analyses that evidenced two well-supported clades, one (0.95 BPP/93 BS) composed of only Southern Brazilian sequences, including the epitype, and the other (1 BPP/100 BS) with sequences from Argentina, Brazilian Amazon, and Costa Rica (Palacio et al. 2021). However, in our reconstructed multigene phylogeny with a more robust dataset and with more sequences from additional loci (Fig. 1), the species was recovered mostly in a single broad clade, indicating that *F. brasiliensis* specimens could indeed correspond to a single natural species with a broader morphological and genetic plasticity and an even wider distribution than expected, extending from Argentina and up to the Southern USA. This plasticity correlates to the wide variation of the macromorphological characters analyzed in this study, such as: basidiomata form (applanate, circular, infundibuliform, flabelliform, reniform to spathulate), stipe attachment (central, eccentric, lateral to substipitate), pileus size (14–90 × 15–100 mm), and pore size (1–10 × 0.5–1.8 mm). Mating studies, ecological data, sequences from other loci besides ITS and/or nucLSU, and further morphological studies of specimens of *F. brasiliensis* from North America and other subregions of the Neotropical region could help further clarify the limits of this species.

Favolus brunneofibrillosus Zabin & Menolli, sp. nov. Figs. 4, 5, 8b, 13b,e

MycoBank MB851604

Type: BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 23°38'31.3"S 46°37'29.2"W, on fallen decaying log, 15 April 2022, *M.P. Drewinski MPD711* (**holotype** FIFUNGI-273), GenBank: ITS = OR346137; nucLSU = OR351903, Culture: CCIBt 4769.

Etymology: *brunneofibrillosus* (Latin), referring to the dark brownish and the appressed to reticulate fibrillose pileus surface.

Diagnosis: *Favolus brunneofibrillosus* is morphologically and phylogenetically close to *F. yanomamii* but it differs from the latter mainly by the dark brownish or pale cream, when

drenched, and an irregularly pubescent pileus surface, not hirsute, which can also have minutely appressed fibrils, forming dark strips in a radial or reticulate pattern, and larger basidiospores (m = 10.0 × 3.5 µm).

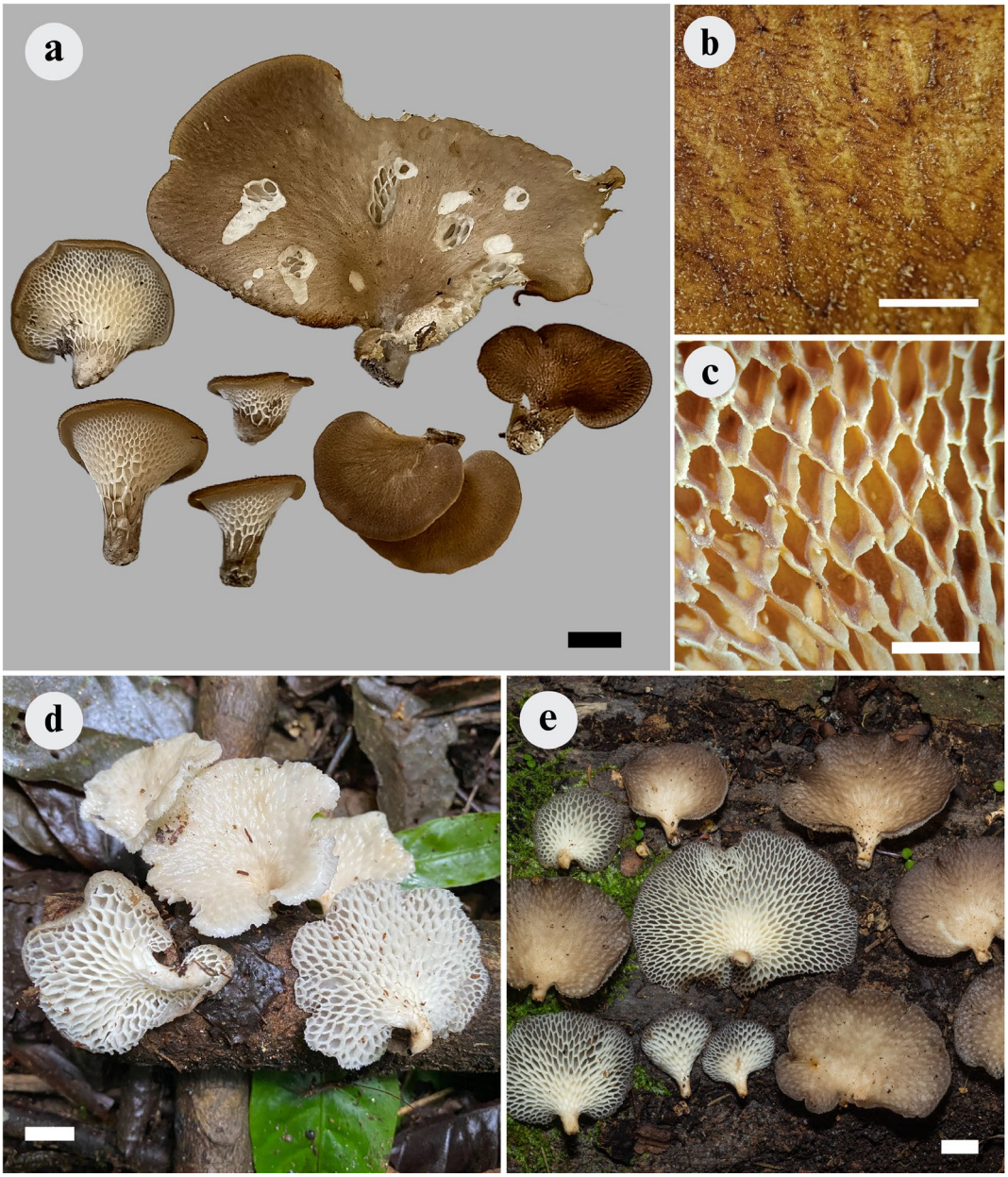

Fig. 4 *Favolus brunneofibrillosus.* a, d, e. Basidiomata. b. Details of the pileus surface. c. Details of the pores. a–c. MPD711, holotype. d. DAZ09. e. DAZ244. Black bars and white scale bars = 1 cm. Photos: a–c, e. D.A. Zabin; d. N. Menolli Jr.

Basidiomata annual, laterally stipitate to rarely substipitate, gregarious to clustered. Pileus 15–88 mm wide, 10–65 mm from the base to the margin; reniform, flabelliform to spathulate; pileus surface irregularly pubescent towards the margin, composed of short and irregular hairs that are more densely distributed and hispid towards the stipe, sometimes with minutely appressed fibrils, forming minute dark to brown strips in a radial to somewhat reticulate pattern, some fibrils fascicles recurved or erect resembling small bristles, tessellate, more towards the margin; dark brown (6F7) to greyish orange (5A5) or greyish brown (6E5) towards the stipe, sometimes greyish yellow (3B3) from the center to the margin; margin entire,

acute, equal to sometimes crenate or undulate, sometimes deflexed; context of the pileus less than 0.1 mm thick, yellowish white (2A2). Pore surface yellowish white (1A2–4A2) or cream to yellowish brown (5E8) when dry; pores 0.8–4 × 0.3–2.2 mm, larger when closer to the base, angular, radially elongated, decurrent; dissepiments entire to lacerate; tubes 1–4 mm long, concolorous with the pores. Stipe 4–10 × 5–7 mm, cylindrical, solid, surface densely pubescent to hispid, yellowish brown (5F7) to greyish brown (5D3).

Hyphal system dimitic with generative hyphae and skeletal-binding hyphae. Generative hyphae 2.0–3.0 µm wide, with clamp connections or simple septa, hyaline, thin-walled, rare, more frequent in younger basidiomata. Skeletal–binding hyphae up to 8.8 µm wide, thick-walled, flexuous, moderately to frequently branched, nondextrinoid, abundant. Hyphal pegs frequent or rarely absent, up to 200 µm long, composed of generative hyphae. Pileipellis a trichoderm composed of projected fascicles (40–200 × 30–100 µm) of generative, erect, clamped, and hyaline hyphae, 3.0–6.0 µm wide. Basidia 22–32 × 5.0–7.0 µm, clavate, 2- or 4-sterigmate; basidioles 17.0–23 × 4.0–6.0 µm, clavate. Basidiospores (7.2–)8.4–12.2(–12.6) × (2.2–)2.4–4.5 µm (m = 10.0 × 3.5 µm), Q = 2.00–4.15 (m = 2.83; n = 350/2/5), cylindrical, thin-walled, smooth, hyaline, frequently with 1 or 2 guttules, IKI–, CB–.

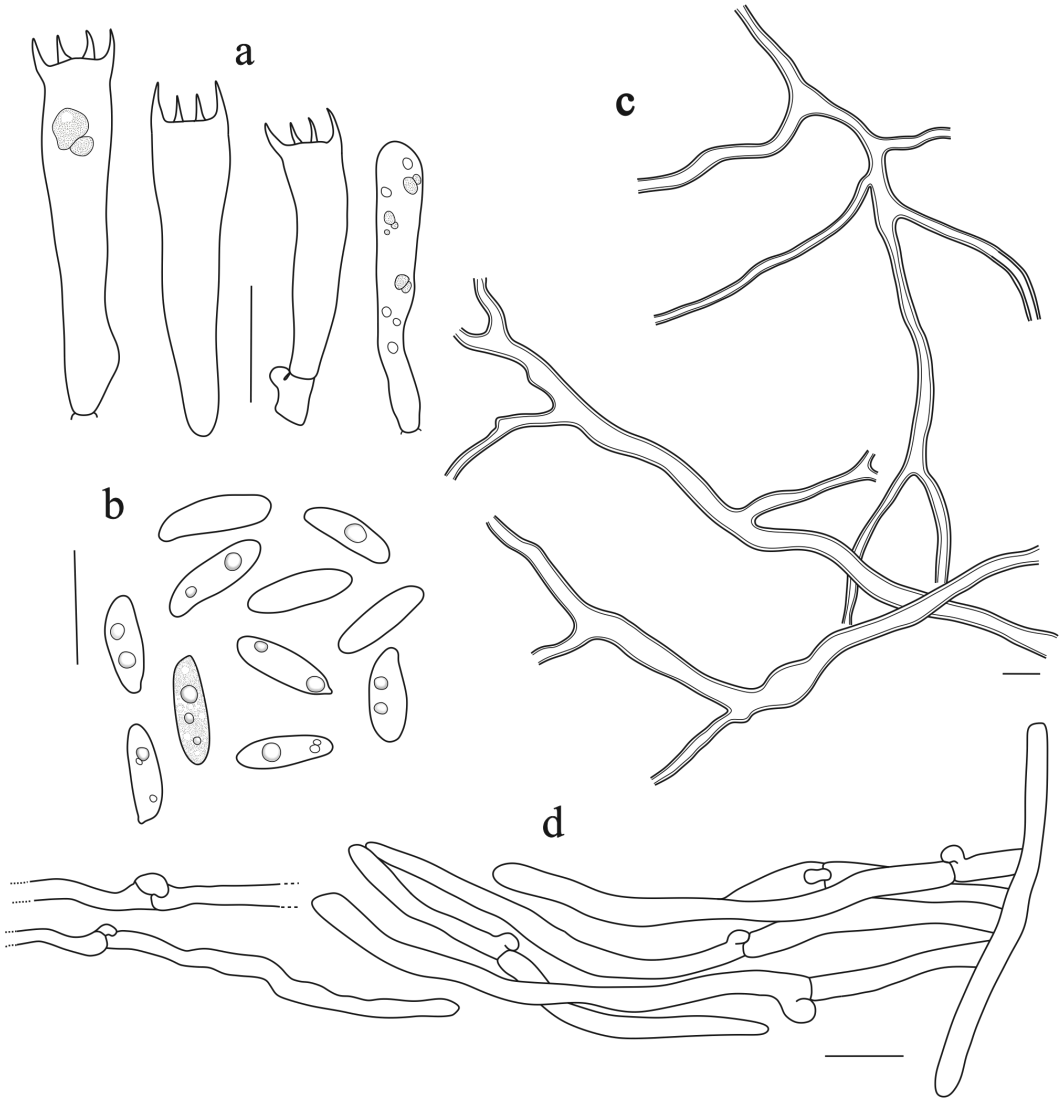

Fig. 5 Microcharacters of *F. brunneofibrillosus* (MPD711, holotype). a. Basidia and basidioles. b. Basidiospores. c. Skeletal-binding hyphae. d. Generative hyphae. Scale bars = 10 µm. Line drawings by C.C. Nascimento.

Culture: Mycelium growing prostrate to the medium, submerged mycelium absent. Aerial mycelium felty to thinly cottony, slightly appressed to the medium, pale to yellowish white, concentrically zonate, sometimes with a distinct inner zone close to the center that is more floccose, zonations ranging from light yellow to greyish yellow or slightly purplish, reverse unaltered. Generative hyphae 3.0–6.0 µm wide, firm-walled, hyaline, with frequent clamp connections closer to the marginal zone; skeletal hyphae 1.5–4.5 µm wide, thick-walled, present only on the inner mycelium zone. Arthroconidia abundant across the whole culture and composed of fragmented hyphae of variable length, which can also be rather ellipsoid with thickened walls.

Ecology and distribution: On fallen decaying logs of unidentified angiosperms in Dense Ombrophilous Forests. Known from the Atlantic and Paraná Forest provinces, January to April, August.

Specimens examined: BRAZIL: RIO DE JANEIRO: Paraty, Parque Nacional da Serra da Bocaina, trail to Sítio São José, on a decaying fallen branch, 11 February 2021, *D.A. Zabin DAZ09* (FIFUNGI-267); SÃO PAULO, São Paulo, Parque Estadual das Fontes do Ipiranga, 23°38'28.5"S 46°37'25.2"W, on a decaying unidentified tree stump, 18 February 2022, *D.A. Zabin DAZ139* (FIFUNGI-251); São Paulo, Parque Estadual das Fontes do Ipiranga, 23°38'31.3"S 46°37'29.2"W, on a decaying log, 13 August 2023, *D.A. Zabin DAZ244* (FIFUNGI-278); ibid., on fallen decaying log, 15 April 2022, *M.P. Drewinski MPD711* (FIFUNGI-273); Ubatuba, near Cachoeira da Lagoinha, 26 January 2022, *N. Menolli Jr NMJ441* (FIFUNGI-150).

Culture examined: CCIBt 4769, obtained from collection *MPD711* (Brazil, São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 23°38'31.3"S 46°37'29.2"W) and isolated by M.P. Drewinski on 15 April 2022.

Comments: The dark brownish, the pubescent and minutely appressed-fibrillose pileus surface composed of darkish brown fibrils in a radiating to reticulate pattern along with the radially elongated pores and a trichodermial pileipellis are some of the diagnostic characters that differentiates *F. brunneofibrillosus* from other known species of *Favolus.* Even though, environmental conditions, such as rain, could lighten up and wash some of the pileus surface features, such as the color and the fibrillose pileus surface, giving the pileus a cream to greyish color, as noted for some of our collections (Fig. 4d). *Favolus yanomamii* forms a sister clade to *F. brunneofibrillosus* (Fig. 1), and both species shares the brownish laterally stipitate basidiomata with radially elongated pores, the presence of generative hyphae with clamp connections and a trichodermal pileipellis (Palacio et al. 2021). However, *F. yanomamii* mainly differs by the lighter brownish to greyish yellow and a more hispid or hirsute pileus surface, shorter tubes (up to 0.2 vs 1–4 mm) and smaller basidiospores (m = 8.1 × 2.9 vs 10 × 3.5 µm). *Favolus radiatifibrillosus* described from Brazil also has a fibrillose pileus surface and radially elongated pores like *F. brunneofibrillosus* but differs by the more pronounced radially arranged appressed-fibrillose surface with a papery texture and the yellowish-brown to brown color of the pileus, smaller pores (0.8–1.5 × 0.3–0.8 mm), and microscopically by the pileipellis as a rectocutis and smaller basidiospores (m = 8.0 × 3.3 vs 10.0 × 3.5 µm) (Palacio et al. 2021). *Favolus tessellatulus* (Murrill) Sacc. & D. Sacc., originally described from Cuba as *Hexagonia tessellatula* Murrill (Murrill 1904), which has been barely reported since its description (Subero 1988), shares with *F. brunneofibrillosus* the laterally stipitate basidiomata with radially elongated pores together with a somewhat pubescent surface and similar average basidiospore size (m = 10 × 3 µm) but differs by the smaller basidiomata (pileus 20–40 × 10–30 mm), which

are white and with a more obvious tessellate surface (Murrill 1904). Additionally, Fidalgo (1976) identified a collection (Prance, Fidalgo et al. 21318) from Roraima state in the Brazilian Amazon as *Favolus brunneolus* Berk. & M.A. Curtis (Berkeley & Curtis, 1868) and reported that the Sanöma group of the Yanomami people make use of its basidiomata as food. *Favolus brunneolus,* however, is now currently accepted as a synonym of *Echinochaete brachypora* (Mont.) Ryvarden based on the presence of spinulose setoid elements on the hymenium in the type specimen (Borges da Silveira & Wright, 2005; Ryvarden, 1984). Based on the photographs in Fidalgo (1976), the description accompanying the voucher deposited at the fungarium INPA (INPA-Fungos 45297), "Pileus dark brown above with hairs forming radial striations, white beneath with pores decurrent onto stipes, pores elongate, hexagonal, margin sterile, continuous, inflexed, 1.5–3.5 × 2.7-5.5 × 0.1–0.7 cm", and the lack of setoid elements on the voucher (SP112924!) deposited at the fungarium SP, which is likely a duplicate of the material deposited at INPA, this collection identified as *F. brunneolus* could actually correspond to *F. brunneofibrillosus* described here. Unfortunately, the SP112924 voucher was in a poor condition, and no additional diagnostic characters besides the absence of the setoid elements could be noted. Additional collections from the locality where these specimens were found could help further elucidate the potential distribution of *F. brunneofibrillosus* in the Brazilian Amazon and new ethnomycological investigations should be carried out to clarify its use for food by the Yanomami people. Still, the first author ate half of a raw basidiome, and he and his friend also ate 10 g of fresh basidiomata (eight medium sized basidiomata) that were sautéed in olive oil, with minced garlic, salt and white pepper. The result was very crunchy, and both found it to be very tasty, resembling fried bacon or pancetta. No adverse reactions were noticed, indicating its edibility.

Favolus glaucovelutinus Zabin & Menolli, sp. nov. Figs. 6, 7, 8c

MycoBank MB851605

Type: BRAZIL: São Paulo: Itirapina, Cachoeira do Saltão, 22°23'22.7"S 47°53'17.6"W, on fallen decaying log of unidentified angiosperm, 14 Jan 2023, *D.A. Zabin DAZ207* (**holotype** FIFUNGI-259) GenBank: ITS = OR353452; nucLSU = OR351905; *TEF1* = OR735316.

Etymology: *glaucovelutinus* (Latin), referring to the cream to greyish yellow and the densely pubescent to velutinous pileus surface.

Diagnosis: *Favolus glaucovelutinus* is phylogenetically close to *F. rugulosus* and is macromorphologically distinct from the latter mostly by the smaller basidiomata, cream yellowish to greyish yellow pileus surface, which is densely pubescent to velutinous with very short white hairs and tesselate towards the margin, and micromorphologically by the trichodermal pileipellis and much larger basidiospores (m = 11.2 × 4.2 vs 9.6 × 3.6 µm).

Basidiomata annual, laterally stipitate, gregarious. Pileus 20–60 mm wide, 18–45 mm from the base to the margin, 4 mm thick; reniform to spathulate; surface densely pubescent to velutinous, composed of short, irregular to suberect white hairs that are more densely distributed towards the stipe, tessellate closer to the margin; yellowish white (1A2) or pale yellow to pale orange (4A3–5A3) towards the margin to greyish yellow (1B4–4B3) or greyish to brownish orange (5B4–5C4) towards the base; margin entire, strongly deflexed when dry; context less than 0.1 mm wide, yellowish white. Pore surface yellowish white to white (1A2 to 1A1); pores 1–4.5 × 0.5–1.5 mm, angular, radially elongated, slightly decurrent; tubes up to 4 mm long, concolorous

with the pores. Stipe 2–10× 1.5–4 mm, cylindrical, solid, surface densely pubescent, yellowish white to greyish yellow (2A1 to 1B3).

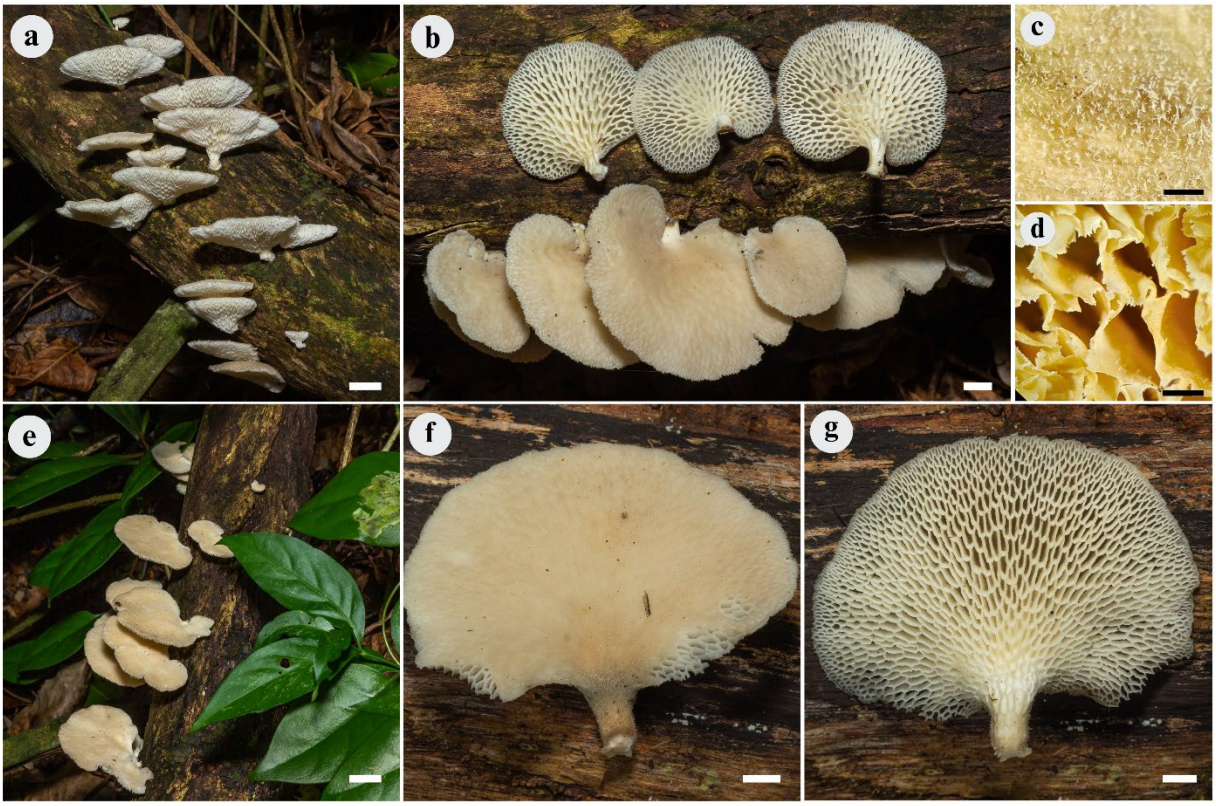

Fig. 6 *Favolus glaucovelutinus*. a, b, e–g. Habit and basidiomata. c. Details of the pileus surface. d. Details of the pores. a-e. DAZ207, holotype. f-g. DAZ270. Black bars = 0,5 cm and white scale bars = 1 cm. Photos: D.A. Zabin.

Hyphal system dimitic with generative and skeletal–binding hyphae. Generative hyphae 1.5–4.0 µm wide, with scattered clamp connections, hyaline, thin-walled, rare. Skeletal–binding hyphae 2.0–8.0 µm wide, thick-walled, flexuous, moderately branched, hyaline, nondextrinoid, abundant. Hyphal pegs 72–130 × 24–54 µm, frequent, composed of generative hyphae. Pileipellis a trichoderm, composed of projected fascicles (50–175 × 15.0–55 µm) of generative, clamped, and hyaline hyphae, 4.5–6.0(–8.0) µm wide. Basidia 26–30 × 5.5–8.0(–10.0) µm, clavate, 2- or 4-sterigmate; basidioles 16.0–24 × 5.0–9.0 µm, clavate. Basidiospores (8.5–)9.6–13.0(–13.3) × (–3.3)3.6–4.6(– 5.2) µm (m = 11.2 × 4.2 µm), Q = (2.1–)2.4–3.1(–3.4) (m = 2.70; n = 100/4/1), cylindrical to slightly bacilloid, hyaline, smooth, guttulate, IKI–, CB–.

Ecology and distribution: On fallen decaying logs of unidentified angiosperms in humid Seasonal Semideciduous Forest fragments. Known from the Brazilian subregion in Paraná Forest province. November to January.

Specimens examined: BRAZIL: SÃO PAULO: Itirapina, Cachoeira do Saltão, 22°23'22.7"S 47°53'17.6"W, on fallen decaying log of unidentified angiosperm, 14 Jan 2023, *D.A. Zabin DAZ207* (FIFUNGI-259); Limeira, Chácara Vale Verde, 22°34'10.3"S 47°20'34.9"W, on decaying fallen log of unidentified angiosperm, 29 November 2023, *D. A. Zabin 270* (FIFUNGI-320).

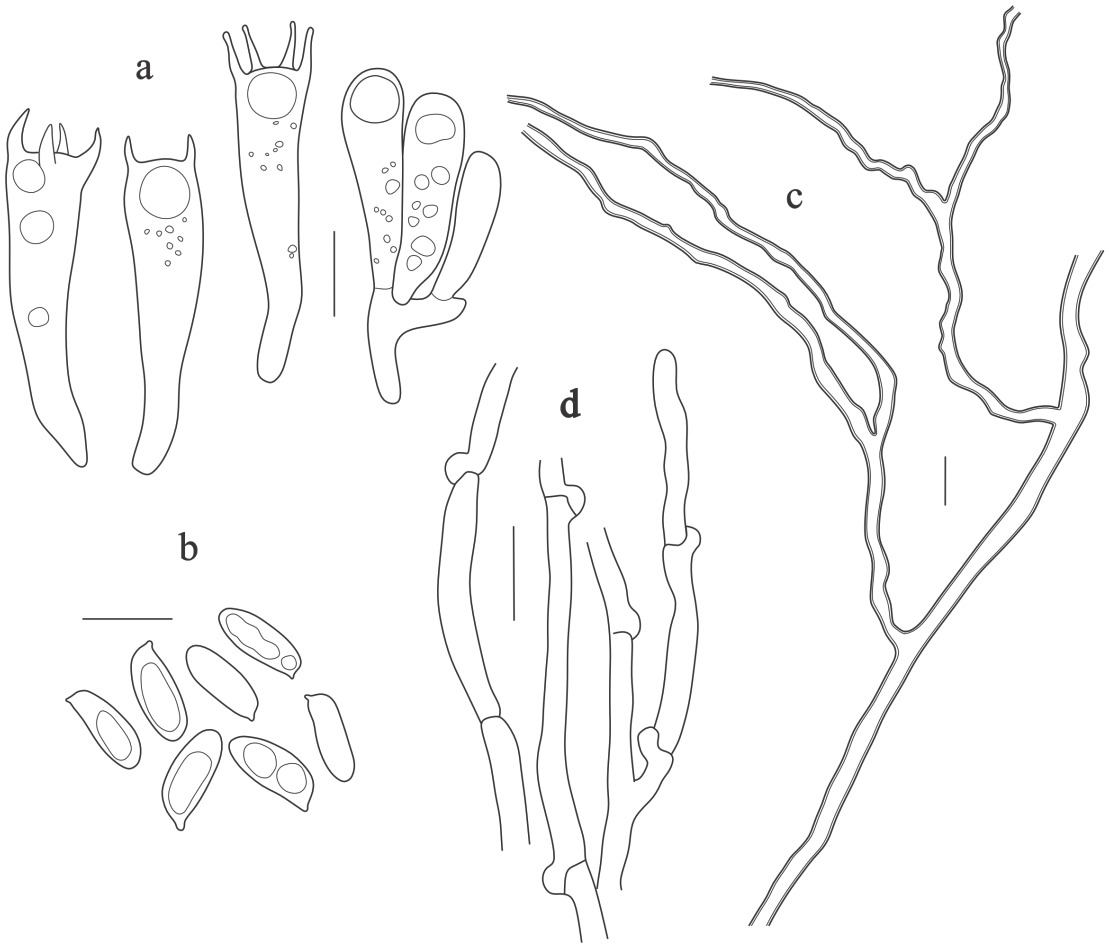

Fig. 7 Microcharacters of *F. glaucovelutinus* (DAZ207, holotype). a. Basidia and basidioles. b. Basidiospores. c. Skeletal-binding hyphae. d. Generative hyphae. Scale bars = 10 μm. Line drawings by C.C. Nascimento.

Comments: *Favolus glaucovelutinus* shares the trichodermal pileipellis with *F. yanomamii* (Palacio et al. 2021) and *F. brunneofibrillosus,* but these last two species are positioned in the /yanomamii clade, while *F. glaucovelutinus* is positioned in the /rugulosus clade (Fig. 1). *Favolus glaucovelutinus* also differs from them because of its densely pubescent to velutinous pileus surface composed of very short white hairs, which when dry gives the pileus surface a whitish fuzzy aspect, almost looking like it was contaminated with mold. Additionally, *F. glaucovelutinus* have larger basidiospores (m = 11.2 × 4.2 μm) than *F. brunneofibrillosus* (m = 10 × 3.5 μm) and *F. yanomamii* (m = 8.1 × 2.9 μm, Palacio et al. 2021). Furthermore, *F. rugulosus,* which is phylogenetically close, shares with *F. glaucovelutinus* the laterally stipitate basidiomata with radially elongated pores, the pubescent pileus surface, and microscopically the trichodermal pileipellis, but differs from *F. glaucovelutinus* by the larger and more robust basidiomata (20–128 × 28–85 vs 20–60 × 18–45 mm) with bigger pores (2–6.0 × 0.5–3.2 vs 1–4.5 × 0.5–1.5 mm) and less lacerated dissepiments, the whitish or darkly greyish and more irregularly pubescent pileus surface that is wrinkled to rugulose when dry, and microscopically by the smaller basidiospores (m = 9.6 × 3.5 vs 11.2 × 4.2 μm) and the pileipellis that can vary from a plagiotrichoderm to a trichoderm. *Favolus subcaperatus* (Murrill) Sacc. & Traverso, originally described as *Hexagonia subcaperata* Murrill based on a specimen from Jamaica (Murrill 1907), has been barely reported since its description (Fidalgo, 1976) and has no additional collections from the type locality but it has been considered a

synonym of *F. tenuiculus* (Gomes-Silva et al. 2012). The first shares with *F. glaucovelutinus* the pileus that is somewhat pale-ochraceous and distinctly pubescent, with a margin that is strongly deflexed when dry and the stipe that is also pubescent but differs by the larger basidiomata (pileus 40–60 × 50–80 vs 18–45 × 20–60 mm) with a flabelliform pileus and non-tessellate surface (Murrill 1907). Based on the original description of *Hexagonia subcaperata* (Murrill 1907), it could indeed correspond to another distinct species of *Favolus* and new collections from the type locality should be carried out to further elucidate the morphological concept of this species.

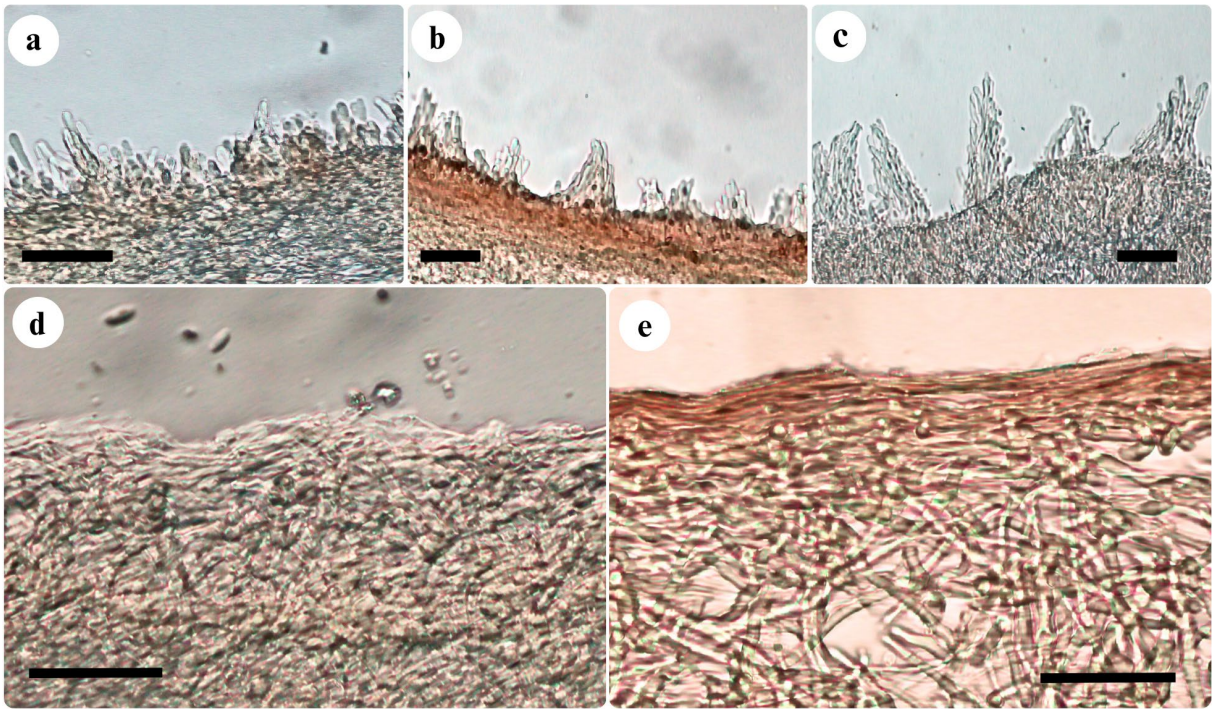

Fig. 8 Pileipellis from the studied *Favolus* spp. a. *Favolus rugulosus* (DAZ79). b. *Favolus brunneofibrillosus* (MPD711, holotype). c. *Favolus glaucovelutinus* (DAZ207, holotype). d. *Favolus brasiliensis* (DAZ70). e. *Favolus radiatifibrillosus* (MPD579). Black scale bars = 50 µm.

Favolus laetiporoides (Vlasák & Ryvarden) Zabin & Menolli, comb. nov.

MycoBank MB851606

Basionym: *Polyporus laetiporoides* Vlasák & Ryvarden, Syn. Fung. 42:32 (2020)

Complete description: see Vlasák & Ryvarden, 2020.

Comments: The species was originally described from Costa Rica and is macromorphologically characterized by the laterally stipitate basidiomata with a semicircular, pale tan or pale brown pileus, up to 30 mm wide and 3 mm thick, with a faintly radially fibrillose surface and tiny erect dark tufts of hyphae, more towards the base, small circular pores, 7–8 per mm, and a short stipe 4 × 5 mm (Vlasák & Ryvarden, 2020). Micromorphologically, it is characterized by a dimitic hyphal system, generative hyphae with scattered clamp connections, skeletal hyphae arboriform, hyaline, solid, up to 7 µm wide in the basal part, sparingly branched, the pileipellis as a cutis, and basidiospores [6–8(–9) × 2–2.5(–3) µm] (Vlasák & Ryvarden, 2020). The

recently described *Favolus pseudogrammocephalus*, known from Brazil and Colombia (Palacio et al. 2021), is similar to *F. laetiporoides* mainly by the small round pores, the pileipellis as a rectocutis, and the basidiospore size range (6–7.5 × 2–3 µm, in *F. pseudogrammocephalus*), but the first differs by the larger basidiomata (90 × 70 × 5 mm), with a greyish orange pileus surface when fresh, which is radially lined and glabrous, bigger pores (4–6 per mm), longer tubes (up to 2 mm long), and microscopically by the generative hyphae with simple septa on the studied collections (Palacio et al. 2021). The inclusion in our phylogenetic analysis of the ITS sequence from the type specimen of *Polyporus laetiporoides* (Vlasák 1704/13) deposited in GenBank (MN272352) resulted in the recovery of the species within the genus *Favolus,* forming a sister branch to *F. ianthinus and F. pseudogrammocephalus* (Fig. 1) nested in the /ianthinus clade, which also includes *F. elongoporus* and *F. radiatifibrillosus*. Based on the original morphological description of *P. laetiporoides* and phylogenetic positioning of the sequence of the type specimen, we propose its combination in *Favolus*.

Favolus radiatifibrillosus Palacio & R.M. Silveira, Mycologia, 113(4):759–775 (2021) Figs. 8e, 9,10

MycoBank MB834611

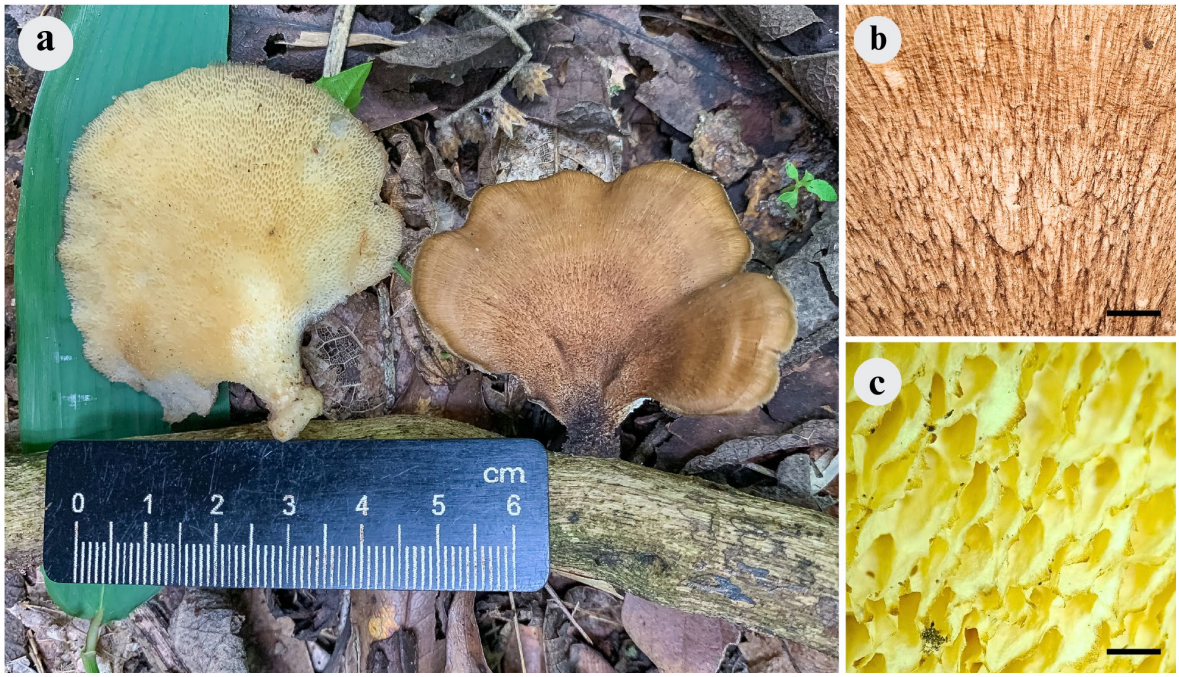

Fig. 9 *Favolus radiatifibrillosus* (MPD579). a. Basidiomata. b. Details of the pileus surface. c. Details of the pores. Photos: a. Tamile R. Santos; b, c. D.A. Zabin. Black scale bars = 0,5 cm.

Basidiomata annual, laterally stipitate, gregarious. Pileus 48–58 mm wide and 38–42 mm from the base to the margin, 4 mm thick; flabelliform to reniform; surface radially appressed–fibrillose with a papery texture; yellowish brown (5D7) to brown (5E4); margin entire, decurved; context 1–3 mm thick, light yellow (3A4) to pale yellow (2A3). Pore surface pale orange (5A3) to yellowish white (2A2); pores 0.6–1.5 × 0.4–0.6 mm, angular, radially elongated, decurrent; dissepiments lacerate; tubes up to 1 mm long, concolorous with the pores. Stipe 5–12 × 6–9 mm, solid, cylindrical, concolorous with the pileus, densely covered with dark irregular to suberect fibrils, and with a thick discoid base.

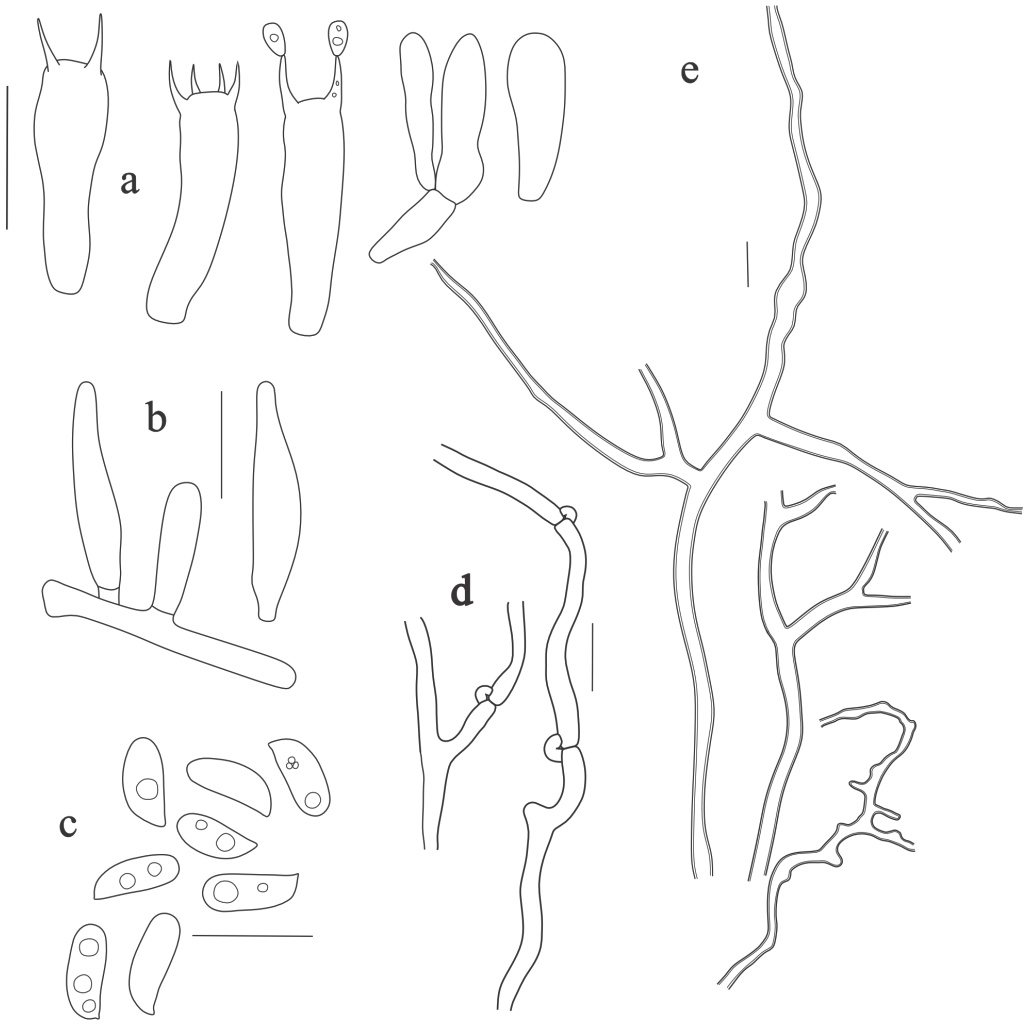

Fig. 10 Microcharacters of *F. radiatifibrillosus* (MPD579). a. Basidia and basidioles. b. Cystidioles. c. Basidiospores. d. Generative hyphae. e. Skeletal-binding hyphae. Scale bars = 10 µm. Line drawings by C. C. Nascimento.

Hyphal system dimitic with generative hyphae and skeletal-binding hyphae. Generative hyphae 2.0–5.5 µm, with simple septa or clamp connections, hyaline, thin-walled, rare. Skeletal–binding hyphae 2.5–7.5 µm, thick-walled to solid, flexuous, branched, hyaline, nondextrinoid, abundant. Hyphal pegs up to 60 µm long, rare. Pileipellis a rectocutis, 8.0–36 µm thick, composed of generative, repent, and hyaline hyphae, 2.5–4.5 µm wide. Cystidioles present, subcylindrical to subulate or sublageniform, 14–22 × 2.8–4.5 µm, frequent. Basidia 16.0–26 µm, clavate, 4-sterigmate; basidioles 14.0–18.0 × 4.0–6.0 µm, clavate. Basidiospores (6.9–)7.1–9.2(–10) × 3.0–3.8(–4.5) µm (m = 8.05 × 3.20 µm), Q = (2.1–)2.3–2.8(–2.92) (m = 2.54; n = 60/1/2), cylindrical, thin-walled, smooth, hyaline, frequently with one or more guttules, IKI–, CB–.

Ecology and distribution: On fallen branches of unidentified angiosperms. Known from Chacoan subregion in Araucaria Forest provinces and also from Atlantic, Caatinga, and Parana Forest provinces and from the Brazilian subregion in Pantepui Province (Palacio et al. 2021). January to April and August to September.

Specimens examined: BRAZIL: BAHIA: Mucugê, Parque Nacional Chapada Diamantina, 12°46′17.8″S, 41°27′00.4″W, 24 Sep 2016, *G. Alves-Silva* 940 (FLOR68429 –

Paratype); SÃO PAULO: Amparo, Monte Alegre, 30 Mar 1943, *M. Kuhlmann* 476 (SP-Fungi 49734 – Paratype); Campos do Jordão, Parque Estadual de Campos do Jordão, Celestina Trail, on a decaying fallen branch, 22 January 2020, *M.P. Drewinski MPD579* (FIFUNGI-101).

Comments: Our collection matches the diagnostic macromorphological features for *Favolus radiatifibrillosus*, a recently described species known from the Brazilian Amazon, Southern Brazil and the Caatinga region from Northeastern Brazil (Palacio et al. 2021), such as the laterally stipitate basidiomata with brownish and radially appressed-fibrillose pileus surface with a papery texture, stipe with a thick discoid base and the angular and radially elongated small pores (Palacio et al. 2021). Microscopically, our collection also agrees with most of the diagnostic characters from the holotype description, such as the pileipellis as a rectocutis and basidiospores size (m = 8.05 × 3.20 µm), which also fall within the range for the species (8–9 × 2.5–3.5) (Palacio et al. 2021). However, some differences were found in our collection, such as the presence of generative hyphae with simple septa or with clamp connections, and the presence of cystidioles that were not reported in the original description. Unfortunately, we couldn't examine the holotype of *F. radiatifibrillosus* because it is not available for loan. In the examined paratypes, we couldn't find generative hyphae with clamp connections nor the presence of cystidioles, even though a note accompanying the voucher FLOR68429 remarked the presence of cystidioles. The voucher FLOR68429 had only a very small basidiome left and it was sterile, it is possible that there was a bigger basidiome that was previously examined and later lost, as there was an additional stipe attached to the substrate without the pileus. Our dried specimens are macromorphologically very similar to the other examined specimens and the holotype, based on photographs of the dried voucher (ICN139554) kindly shared to us. We were able to generate sequences for our collection (ITS, nucLSU and *TEF1*) and also ITS sequences for the two examined paratypes. In our multigene phylogeny, our specimen was recovered along with the two paratype sequences in a single distinct clade nested within the /ianthinus clade (Fig. 1), and the ITS sequences were 99.8% identical which corroborates the interpretation of them corresponding to the same species.

Favolus rugulosus Palacio & R.M. Silveira, Mycologia, 113(4):759–775 (2021) Figs. 8a, 11, 12, 13c,f

MycoBank MB834612

Basidiomata annual, laterally stipitate, gregarious. Pileus 20–128 mm wide, 28–85 mm from the base to the margin, up to 4 mm thick; reniform, flabelliform to spathulate; surface pubescent, more towards the stipe, composed of weak hairs of variable length, frequently tessellate closer to the margin and irregularly wrinkled or rugulose when dry; white (1A1) or greyish yellow (4B3) to yellowish grey (4B2) towards the base and pale grey (1B2) or brownish grey (4D2) to grey (4E2) towards the margin; margin entire, acute, sometimes becoming yellowish brown to brown (5B6 to 6E8) when dry; context of the pileus mostly less than 1 mm thick, pale yellowish (2A2). Pore surface white (1A1) to pale yellow (2A3); pores 1–6 × 0.5–3.2 mm, angular, radially elongated, decurrent; dissepiments lacerate; tubes 1–3 mm long, concolorous with the pore surface. Stipe 4–20 × 4–12 mm wide, cylindrical, solid; surface densely pubescent to hirsute; white (1A1) to greyish yellow (2B3) or greyish brown (5F3) towards the base.

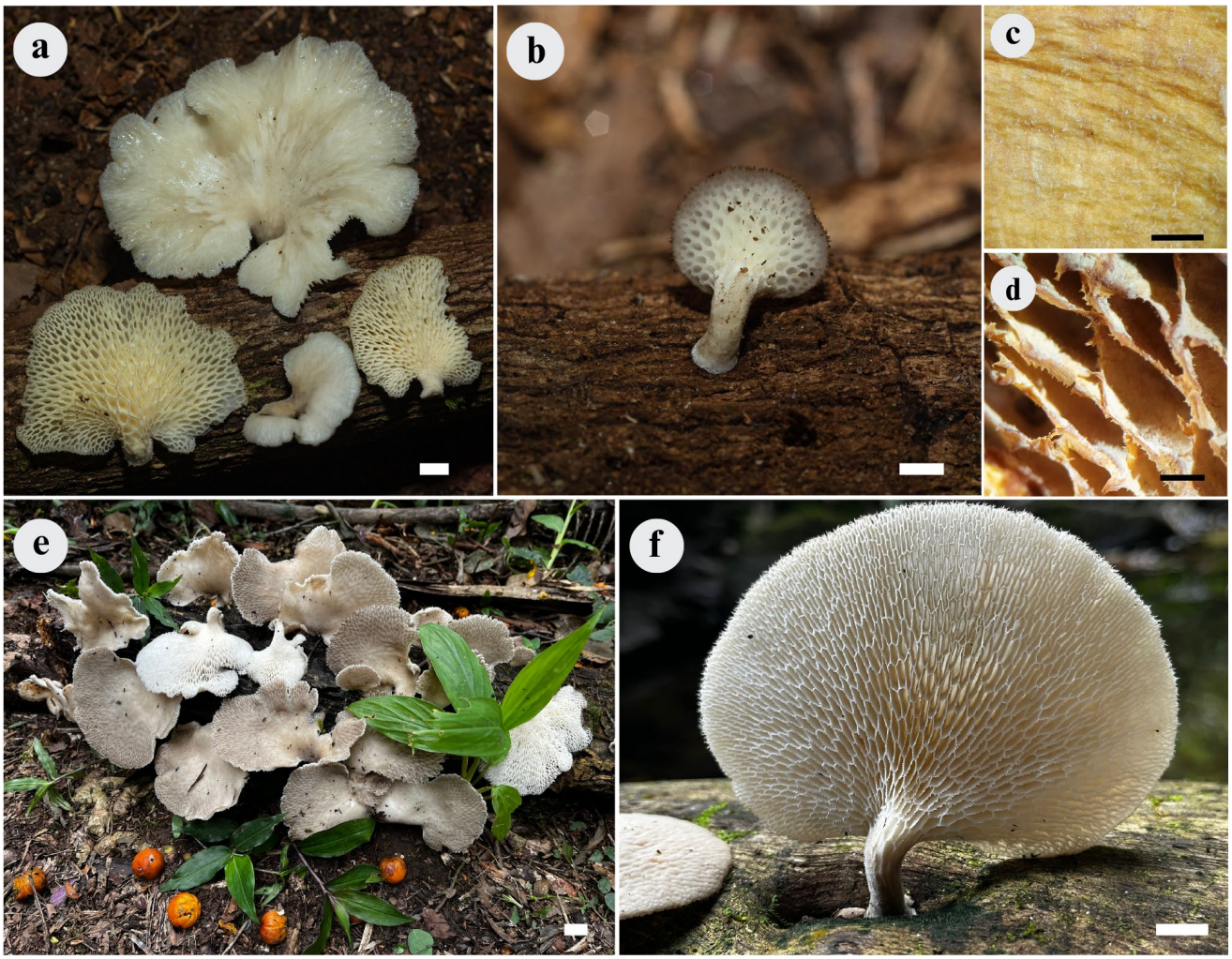

Fig. 11 *Favolus rugulosus.* a,b,e,f. Basidiomata. c. Details of the pileus surface. d. Details of the pores. a–d. DAZ79. e. NMJ459. f. MAC11. Black bars = 0,5 cm and white scale bars = 1 cm. Photos: a–d. D.A. Zabin; e–f. N. Menolli Jr.

Hyphal system dimitic with generative and skeletal-binding hyphae. Generative hyphae 2.0–6.4 µm wide, with clamp connections, hyaline, thin-walled, rare, with some inflated generative hyphae up to 10 µm wide infrequently found in the tube trama. Skeletal-binding hyphae 1.8–9.8 µm wide, thick-walled to solid, flexuous, moderately branched, hyaline, nondextrinoid, abundant. Hyphal pegs 64–122 × 24–40 µm, sparse, composed of generative hyphae. Pileipellis a plagiotrichoderm, composed mostly of basal repent generative hyphae with terminal hyphae projected in short fascicles (18–55 × 6.0–36 µm), or frequently, a trichoderm composed of projected fascicles (98–350 × 24–100 µm) of generative, erect, clamped, and hyaline hyphae, 1.5–6.8 µm wide. Basidia 16–35 × 5.0–8.5 µm, clavate, 2- or 4-sterigmate; basidioles 14.0–28 × 4.8–7.8 µm. Basidiospores 7.0–12(–12.8) × (2.6–)2.9–4.3(–4.5) µm (m = 9.6 × 3.5 µm), Q = 2.1–3.4 (m = 2.80; n = 282/1/8), cylindrical, thin-walled, smooth, hyaline, guttulate, IKI–, CB–.

Culture: Mycelium growing appressed to the medium, submerged mycelium absent. Aerial mycelium initially velvety to felty, thick, white, forming deep orange to dark brown, irregular, thick and hard crusts from the center to the margin, mycelium mat turning entirely crustose with time, sometimes rather zonate, colorless exudates forming on the crust surface. Generative hyphae 2.0–5.0 µm wide, firm-walled, with frequent clamp connections, more abundant in the marginal zone; skeletal hyphae 1.5–3.5 µm wide, thick-walled, flexuous,

branched, rarely septate. Hyphae from the crusts, encrusted with brownish resinaceous matter. Arthroconidia more frequently present in the marginal zone, composed of fragmented hyphae of variable length, which can also be rather ellipsoid with thickened walls.

Ecology and distribution: Growing on various unidentified fallen angiosperm logs, on cultivated *Carya illinoiensis* (*Juglandaceae*) fallen decaying log and on decaying *Ficus* sp. (*Moraceae*) roots. Known from the Brazilian subregion in Magdalena province, Western Ecuador province and in Chacoan subregion of Atlantic, Pampean, and Parana Forest provinces (Palacio et al. 2021, Veloso et al. 2023). September, November to January, March to May.

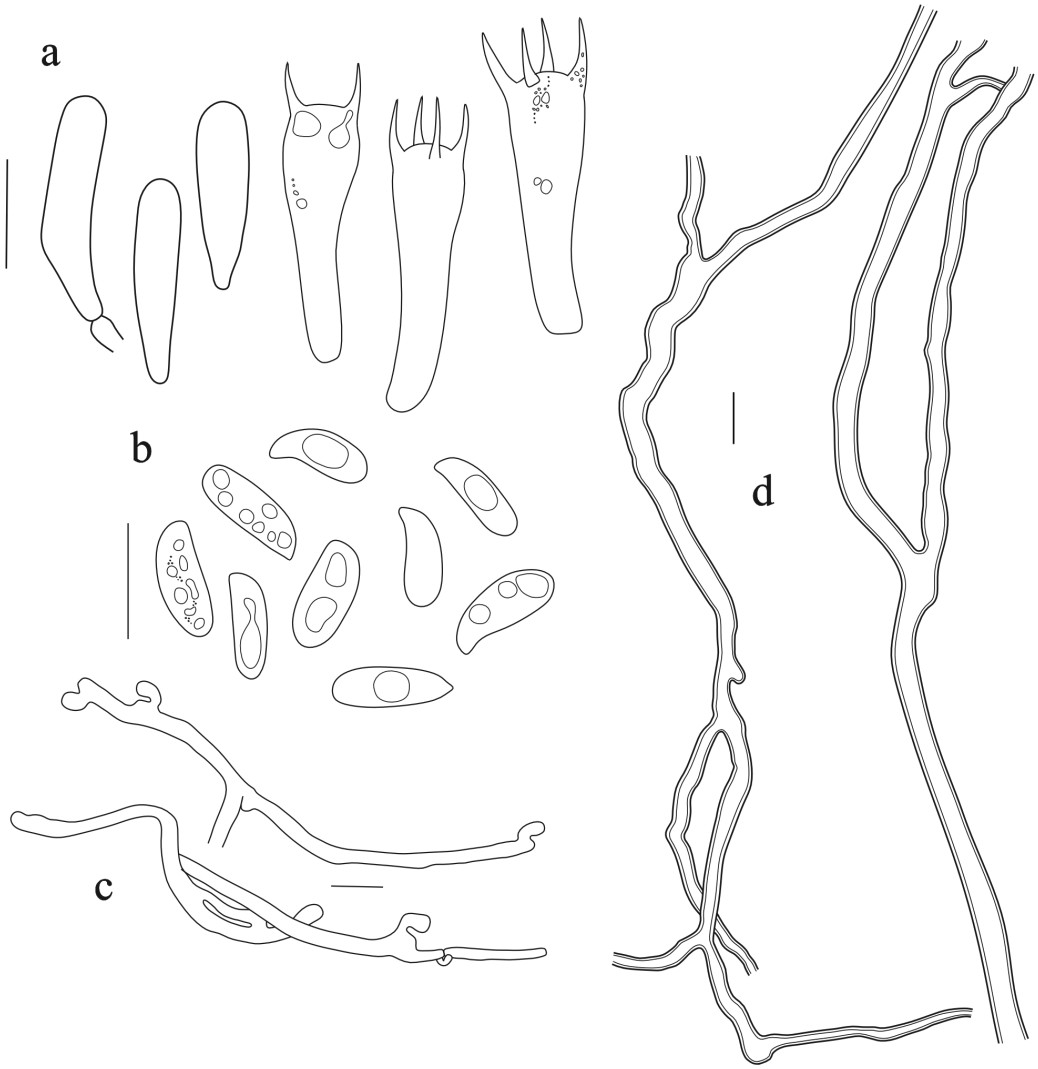

Fig. 12 Microcharacters of *F. rugulosus* (DAZ79). a. Basidia and basidioles. b. Basidiospores. c. Generative hyphae. d. Skeletal-binding hyphae. Scale bars = 10 μm. Line drawings by C.C. Nascimento.

Specimens examined: BRAZIL: SÃO PAULO: Angatuba, Rural Area, on decaying fallen log, 21 October 2022, *L. Trierveiler-Pereira s.n.* (FIFUNGI-260); Cananeia, Parque Estadual da Ilha do Cardoso, Núcleo Marujá, Tapera Trail, on decaying fallen log, 25 September 2018, *M.P. Drewinski MPD240* (SP528743); Caraguatatuba, Parque Estadual da Serra do Mar, Núcleo Caraguatatuba, on a decaying fallen log, 26 January 2022, *D.A. Zabin DAZ79* (FIFUNGI-144); Iporanga, Parque Estadual Turístico do Alto Ribeira, Núcleo Santana, on fallen decaying log, 03 November 2022, *A.C. Morais, MAC11* (FIFUNGI-277); *ibid*.,

24°32’2” S 48°42’7” W, on a fallen decaying log, 05 November 2022, N. Menolli Jr., *NMJ459* (FIFUNGI-202); *ibid*., 24°32’8”S 48°42’10” W, on a fallen decaying log, *NMJ460* (FIFUNGI-276); Limeira, Bairro dos Pires, 22°34'09.3"S 47°20'51.3"W, on decaying *Ficus* sp. roots, 29 December 2022, *D.A. Zabin, DAZ189* (FIFUNGI-253); *ibid.*, Chácara Vale Verde, 22°34' 10.8” S, 47°20'35.77” W, on fallen decaying log, 16 October 2023, *DAZ*255 (FIFUNGI-315); *ibid.*, on fallen decaying log of cultivated *Carya illinoiensis* (*Juglandaceae*), 03 December 2023, *DAZ279* (FIFUNGI-319); São Sebastião, Parque Estadual de Ilhabela, Bonete Trail, on decaying fallen log, 4 December 2020, *N. Menolli Jr. NMJ376* (FIFUNGI-104).

Cultures examined: CCIBt 4784, obtained from the collection *MPD240* (Brazil, São Paulo: Cananeia, Parque Estadual da Ilha do Cardoso, Núcleo Marujá, Tapera Trail) and isolated by M.P. Drewinski on 25 September 2018.

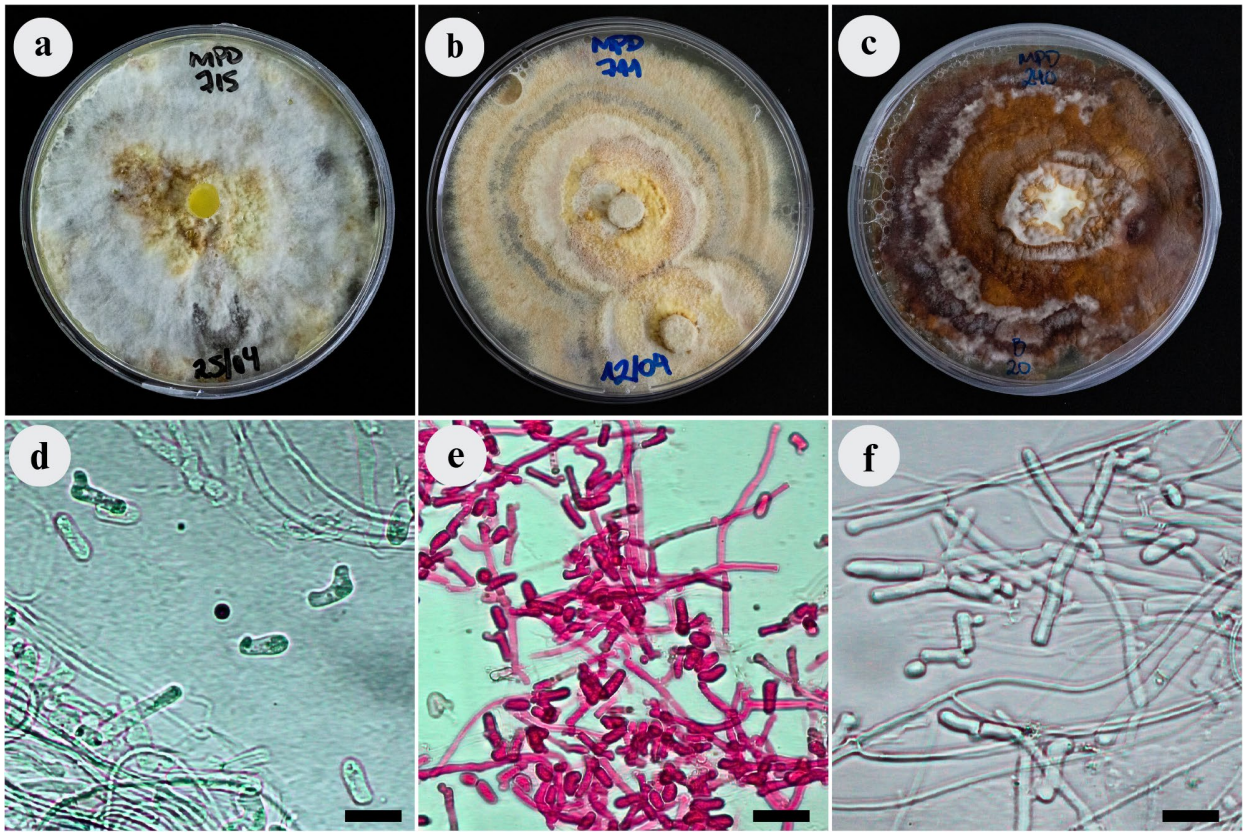

Fig. 13 Macro and microcharacters of pure cultures of *Favolus* spp. grown on PDA at 25 °C. a–c. Pure culture on Pretri dishes 90 mm diam, d–f. Arthroconidia. a, d. *Favolus brasiliensis* (CCIBt 4770). b, e. *Favolus brunneofibrillosus* (CCIBt 4769). c, f. *Favolus rugulosus* (CCIBt 4784). Black scale bars = 25 µm.

Comments: *Favolus rugulosus* was recently described based on collections from Southern Brazil and Colombia (Palacio et al. 2021). Our collections agrees with most of the diagnostic characters from the original description of *F. rugulosus,* such as the pubescent pileus surface composed of short weak yellowish hairs that is irregularly wrinkled or rugulose when dry and the densely pubescent stipe, but differs by the slightly larger basidiospores (m = 9.6 × 3.5 vs 9.0 × 3 µm) (Palacio et al. 2021) and by the pileipellis, which in our collections varied from a plagiotrichoderm to a trichoderm in most of the collections examined, composed of

larger and longer projected fascicles of generative hyphae. Our morphological description of a pure culture (CCIBt 4784) obtained from this species also agrees with a previous cultivation study of *F. rugulosus* that reported the formation of brown hard crusts from the center of the culture (Sanchez-Ocampo et al. 2022). Additionally, we also call attention to the considerable variation on the pileus coloration in our collections, ranging from almost pure white, to brownish grey, yellowish grey, and other shades of grey. Some of the whitish specimens when fresh could even resemble *F. brasiliensis* but differing mainly by the more robust basidiomata with an irregularly pubescent pileus surface without radial striations and larger pores (1–6 × 0.5–3.2 mm vs 1–10 × 0.5–1.8 mm). Basidiomata of this species are regarded as being edible (Palacio et al. 2021; Sanchez-Ocampo et al. 2022). During one of our field surveys, basidiomata of *F. rugulosus* were also eaten in a pizza made along with some other wild edible mushrooms species but they were found to be too leathery, suggesting the need for different cooking techniques.

Key to *Favolus* species from Brazil

1. Pores angular and radially elongated 2

1’. Pores round or irregularly elongated to daedaloid ……..... 8

2. Pilear surface radially striate or radially appressed-fibrillose, glabrous to irregularly pubescent or hispid towards the base; pileipellis as a rectocutis ……..... 3

2’. Pilear surface not radially striate, but pubescent, velutinous, hirsute, minutely fibrillose or with hydnoid protuberances; pileipellis as a plagiotrichoderm or a trichoderm ……..... 4

3. Pilear surface white to pale yellow, radially striate, glabrous to irregularly pubescent or hispid close to the stipe ……..... *F. brasiliensis*

3’. Pilear surface yellowish brown to brown, radially appressed-fibrillose ……... ……..... *F. radiatifibrillosus*

4. Pilear surface with hydnoid protuberances; ……..... *F. biskeletalis*

4’. Hydnoid protuberances absent from pileus surface 5

5. Pilear surface brownish, irregularly pubescent, hirsute or with fibrils arranged in a radial or reticulate pattern ……..... 6

5’. Pilear surface white, cream or greyish, pubescent to velutinous ……..... 7

6. Pilear surface light brown to greyish yellow, hirsute; basidiospores m = 8.1 × 2.9 µm ……..... *F. yanomamii*

6’. Pilear surface dark brown and pale greyish yellow when drenched, irregularly pubescent and minutely appressed-fibrillose; basidiospores m = 10 × 3.5 µm ..... *F. brunneofibrillosus*

7. Pilear surface cream to greyish yellow or pale orange, densely pubescent, velutinous, composed of very short whitish hairs; basidiospores m = 11.2 × 4.2 µm ……..…..…..... *F. glaucovelutinus*

7’. Pilear surface white or greyish, wrinkled or rugulose when dry, irregularly pubescent, composed of short golden hairs; basidiospores m = 9.6 × 3.5 µm ……..... *F. rugulosus*

8. Pores irregularly elongated to daedaloid …….....……..... *F. elongoporus*

8’. Pores regularly round 9

9. Pilear surface greyish orange, radially lined, glabrous ……..... *F. pseudogrammocephalus*

9’. Pilear surface violet-brown to pinkish grey, radially striate, glabrous ..... *F. ianthinus*

Discussion

Our taxonomic study incorporating morphological and multigene (ITS, nucLSU, *TEF1*, and *RPB1*) molecular phylogenetic analyses revealed two new species from the genus *Favolus* from Southeastern Brazil. These two new species, described here as *F. brunneofibrillosus* and *F. glaucovelutinus,* were both recovered in one of the two major clades of the genus that contains only Neotropical species with radially elongated pores. Additionally, the phylogenetic position of *F. radiatifibrillosus* was elucidated based on the first sequences (ITS, nucLSU, and *TEF1*) obtained from a recent collection and also from two paratypes. The inclusion of an ITS sequence from the isotype of *F. elongoporus* also elucidated its phylogenetic position. *Favolus radiatifibrillosus* and *F. elongoporus* were both nested in the /ianthinus clade (Fig. 1) that also contains the Neotropical *F. ianthinus, F. laetiporoides*, and *F. pseudogrammocephalus*, which have regular circular pores. The /ianthinus clade, in turn, is included in the second major clade of *Favolus*, which also includes European and Asian species of the genus that have either angular and radially elongated pores, such as *Favolus niveus* Jun L. Zhou & B.K. Cui, *F. pseudobetulinus* (Murashk. ex Pilát) Sotome & T. Hatt., *F. roseus* Lloyd, and *F. spatulatus* (Jungh.) Lév, or circular pores, like *F. acervatus* (Lloyd) Sotome & T. Hatt., *F. emerici* (Berk. ex Cooke) Imazeki, and *F. pseudoemerici* Jun L. Zhou & B.K. Cui (Sotome et al. 2013; Zhou & Cui, 2017). The position of *F. elongoporus*, which have irregularly elongated to daedaloid pores, in this major clade of *Favolus* was already hypothesized by Palacio et al. (2021) and by the inclusion of the isotype sequence of this species in our analysis, we were able to confirm it. Finally, a better understanding and resolution of the phylogenetic relationship of specimens of *F. brasiliensis* from the Neotropical region was obtained based on new sequences from four different loci (ITS, nucLSU, *TEF1*, and *RPB1*) from various collections of the species from Brazil with additional ITS and/or nucLSU sequences from Argentina, Costa Rica, Honduras and the USA. Thus, it corroborates the interpretation of *F. brasiliensis* as a single broad clade and questioning its status as a species complex according to a previous phylogenetic reconstruction (Palacio et al. 2021).

Our taxonomic investigation recognizes the pilear surface structure, the hymenophore morphology, and the basidiospore size as being the most informative and variable morphological characters for the Neotropical species of the genus. Despite this, some variations of these characters, such as the color and arrangement of the pileus surface and the pore size, were noted in the study from specimens of the same taxa, which could be due to intraspecific phenotypic plasticity or environmental factors. Some environmental factors, such as CO_2 concentration, presence or intensity of light, and relative humidity and temperature are known to cause major alterations on the morphology of basidiomata, including abnormal elongation of the stipe or malformations of the pileus and hymenophore (Sakamoto, 2018). On the other hand, other environmental conditions such as the drenching of basidiomata from heavy rain and subsequent drying can also be associated with some other changes in morphology such as the alteration of pileus surface structure and color, a phenomenon known as hygrophany (Harmaja, 1969). It is known that the absorption of water from rain can dissolve, and wash away water-soluble pigments present on the intracellular space or inside the vacuoles from the hyphae of the pileus of mushrooms (Clémençon, 2004), and thus lightning its color. The rain could even wash fibrillose or veil structures from the surface (Baroni et al. 2020; Esteve-Raventós et al. 2018). Lighter colored and less pubescent or lacking the characteristic fibrillose surface of specimens of *F. brunneofibrillosus* were found after intense rainfall, which could be an explanation for the variation on the pileus surface verified on this species. Given the variability and partial overlap among the limited informative morphological characters for distinguishing species of *Favolus*, we emphasize the importance of incorporating additional molecular, physiological, and ecological data whenever feasible.

Cultures derived from some of the species investigated in this study (*F. brasiliensis*, *F. brunneofibrillosus*, and *F. rugulosus*) exhibit marked morphological distinctions among themselves (Fig. 13). These disparities encompass growth patterns at the marginal zone, coloration, density, texture, and the arrangement of hyphae in the mycelium mat, as well as the development of either crusts or discernible zonal patterns. Microscopically, all cultures from the species examined share certain traits, such as the presence of clamped generative hyphae, thick-walled skeletal hyphae, and presence of asexual structures such as arthroconidia, albeit notably more abundant in the pure culture of *F. brunneofibrillosus*. Our examination of these *Favolus* species in culture underscores the potential of utilizing the distinct morphological attributes of pure cultures within the genus as an additional and reliable method for species identification and delimitation besides the assessment of basidiomata morphology and molecular data.

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Author contribution

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Denis A. Zabin and Cristiano C. Nascimento. The

first draft of the manuscript was written by Denis A. Zabin, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

DNA sequences used in the present study are available in GenBank. Alignments and tree were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S31073). Fungal specimens are stored in public herbaria (FIFUNGI, FLOR and SP).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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CAPÍTULO 2

In vitro mycelium growth and biomass production of wild strains of three edible mushroom species of *Favolus* from the Brazilian Atlantic Forest

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Abstract

Favolus is a genus of polypores that can be recognized by its fleshy and laterally stipitate basidiomata with mostly radially elongated pores and by the white rot decomposition of dead wood from various plant species. The genus is widely distributed in the Neotropical region, with species that besides their ecological importance as wood decomposers are also economically important as wild edible mushrooms used by traditional communities. Some of the known edible species of the genus are abundant in the Brazilian Atlantic Forest and potentially significant for their use as food and for future commercial cultivation. Considering the relative few studies regarding the domestication and cultivation of *Favolus* species, this study aims to evaluate the *in vitro* mycelium growth and biomass production efficiency of wild strains obtained from three edible species of the genus: *Favolus brasiliensis*, *Favolus brunneofibrillosus*, and *Favolus rugulosus*. The effect of different incubation temperatures (20 ºC, 25 ºC, 30 ºC, and 35 ºC) and solid culture media (Potato Dextrose Agar, Malt Yeast Peptone Agar, Yeast Glucose Agar, and Soy Agar) on the growth parameters of the strains were evaluated. The best temperatures for the mycelium growth and biomass production for the studied strains were either 25 °C or 30 °C, and all the strains were able to grow on all tested solid culture media. The wild strain CCIBt4770 of *F. brasiliensis* achieved the best mycelium growth overall, with the best combinations of culture media and temperature being either MYPA or YGA at 25 ºC. For *F. brunneofibrillosus*, the wild strain CCIBt4769 had the best growth either on MYPA or PDA at 25 ºC or 30 ºC. Finally, the wild strain CCIBt4784 of *F. rugulosus* performed at its best when growing on the SOY medium at 30 ºC.

Keywords: *Favolus brasiliensis*; *Favolus brunneofibrillosus*; *Favolus rugulosus*; Mushroom cultivation; Wild edible mushrooms

Introduction

Among the 2.189 known edible mushroom species in the world (Li et al. 2021), around 100 species have been domesticated and over 60 species are commercially cultivated (Li & Xu, 2022). Despite this, only five genera constitute around 85% of the global mushroom market: *Lentinula* Earle as the major cultivated genus, with *Lentinula edodes* (Berk.) Pegler being the only commercially cultivated species of the genus, contributing to about 22% of the global production, followed by *Pleurotus* (Fr.) P. Kumm. (19%), *Auricularia* Bull. (17%), *Agaricus* L. (15%), and *Flammulina* P. Kumm. (11%) (Royse et al. 2017).

In Brazil, the interest for wild and cultivated edible mushrooms is growing, as well as the mushroom production and market. The most recent data points to an annual production of 15.696 tons in the country, corresponding to around 2.35% of the total production in the Americas (Sanchéz et al. 2018). This total production is prevalent in the cultivation of three species: *Pleurotus ostreatus* (Jacq.) P. Kumm. (48%), *Agaricus bisporus* (J.E. Lange) Imbach (33%), and *Lentinula edodes* (13%), with the remaining 6% composed of various species, such as *Agaricus subrufescens* Peck., *Pleurotus djamor* (Rumph. ex Fr.) Boedijn, *P. cornucopiae* (Paulet) Quél., and *P. eryngii* (DC.) Quél. (Sanchéz et al. 2018).

The strains used for the commercial cultivation of these species in tropical areas are mostly from temperate regions (Stamets, 2000), which require optimization of the climatic conditions during their cultivation and that in turn can lead to a higher cost for mushroom growers and can make their cultivation at large scales in hotter climates unsustainable (Vargas-Isla & Ishikawa, 2008). Most strains of mushrooms from tropical areas, however, can grow rapidly and form basidiomata at temperatures of 25 ºC or higher (Thawthong et al. 2014), and so could be produced more efficiently than strains from temperate regions. Additionally, tropical edible mushrooms can also be grown on readily available and cheap agro-industrial residues, such as sawdust, sugarcane bagasse, rice straw, and various other lignocellulosic wastes (Kumla et al. 2020; Omarini et al. 2009).

The Atlantic Forest ecoregion is a globally important biodiversity hotspot harboring many endemic species across multiple taxonomic groups (Myers et al. 2000; Olson & Dinerstein, 2002) and accommodates the greatest diversity of known fungal species in Brazil (Maia et al. 2015). This diversity includes species with biotechnological potential and industrial applications, while also including various edible mushroom species (Hyde et al. 2019; Niego et al. 2021). The domestication of these wild edible mushroom species could provide more efficient and sustainable alternatives for commercial mushroom growers and expand the very restricted mushroom availability in Brazil with local species. The optimization of the temperature and culture media for the best mycelial growth and biomass production are considered one of the first steps in the domestication studies of promising wild edible mushrooms species (Albertó, 2017; Thawthong et al. 2014).

Favolus Fr. (Polyporaceae, Agaricomycetes), also known as honeycomb fungus, is a widely distributed genus of polypores that causes white-rot decomposition of various woody plant species in tropical to subtropical regions and can be macromorphologically recognized by the fleshy and laterally stipitate basidiomata with mostly radially elongated pores (Sotome et al. 2013). Besides its ecological importance, basidiomata of *Favolus* species are consumed as wild edible mushrooms by traditional communities around the world (Boa, 2004; Degreef et al. 2016; De Leon et al. 2013; Flores-Arzú et al. 2012; Gamboa-Trujilo et al. 2019; Ruán-Soto et

al. 2016; Vargas-Isla et al. 2013; Zent et al. 2004). Among them are the Sanöma, the Tootobi, and the Waukás communities of the Yanomami people from the Brazilian Amazon Forest that include basidiomata of *Favolus* species in their diet, such as: *Favolus brasiliensis* (Fr.) Fr., *F. radiatifibrillosus* Palacio & R.M. Silveira, and *F. yanomamii* Palacio & Menolli (Prance, 1972, 1973, 1984; Fidalgo & Prance, 1976; Sanuma et al. 2016; Palacio et al. 2021), with the two latter previously identified as *Polyporus philippinensis* Berk. (Palacio et al. 2021). Additionally, records of edible *Favolus* species in Brazil also include the recently described, *F. brunneofibrillosus* Zabin & Menolli (Zabin et al. 2023) and *F. rugulosus* Palacio & R.M. Silveira (Palacio et al. 2021).

Currently, there are no species of *Favolus* that are commercially cultivated, and domestication studies are very few and far between, even though they were successful on the cultivation of *Favolus* species, such as *F. rugulosus* from Colombia (Sanchez-Ocampo et al. 2022) and Paraguay (Veloso et al. 2023), *Polyporus grammocephallus* Berk. [= *Favolus grammocephallus* (Berk.) Imazeki] from the Philippines (De Leon et al. 2013), and as *Polyporus tenuiculus* (P. Beauv.) Fr. (= *Favolus tenuiculus* P. Beauv.) from Argentina (Omarini et al. 2009) in agro-industrial residues with formation of basidiomata.

Based on wild strains obtained from specimens of *F. brasiliensis*, *F. brunneofibrillosus*, and *F. rugulosus* collected in the Atlantic Forest from Southern and Southeastern Brazil, this study aims to evaluate the *in vitro* mycelium growth and biomass production of these strains in different temperatures (20 ºC, 25 ºC, 30 ºC, and 35 ºC) and solid culture media: Malt Yeast Peptone Agar (MYPA), Potato Dextrose Agar (PDA), Soy Agar (SOY) and Yeast Glucose Agar (YGA).

Materials and Methods

Collections and strain isolation

Specimens of three *Favolus* species were collected in fragments of Dense Ombrophilous Forest and Seasonal Semideciduous Forest types in the Atlantic Forest (Oliveira-Filho & Fontes, 2000; Veloso et al. 1992) from Southeastern Brazil during the most humid months (October to March). Specimen vouchers are deposited at the Fungarium IFungiLab (FIFUNGI) from the 'Instituto Federal de Educação, Ciência e Tecnologia de São Paulo' (IFSP – São Paulo, Brazil), and at the Fungarium SP from the 'Instituto de Pesquisas Ambientais' (IPA– São Paulo, Brazil) (Thiers, 2023, continuously updated).

Cultures were isolated from tissue fragments obtained from the pileus or stipe context of fresh basidiomata that were then cultured and maintained in sterile Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25 ºC with trimestral subculturings. Identification of the strains were carried out based on molecular and phylogenetic analyses from our previous diversity study of *Favolus* from Brazil (Zabin et al. 2023).

Two strains of *Favolus brasiliensis*, one strain of *F. brunneofibrillosus*, and one strain of *F. rugulosus* were obtained and selected for this study. Strains are deposited at the CCIBt Culture Collection from the 'Instituto de Pesquisas Ambientais' (São Paulo, Brazil). Information about the strains and the authentic specimens are in Table 1.

Table 1 Data on the Brazilian wild strains selected for this study.

Strain	Species	Collection	Collection locality	Fungarium voucher
CCIBt4768	*Favolus brasiliensis*	MPD708	Brazil, São Paulo, São Paulo, 'Parque Estadual das Fontes do Ipiranga' (23°38'40.6"S 46°37'27.3"W)	FIFUNGI-271
CCIBt4770	*Favolus brasiliensis*	MPD715	Brazil, Paraná, Guarapuava, private rural area	FIFUNGI-272
CCIBt4769	*Favolus brunneofibrillosus*	MPD711	Brazil, São Paulo, São Paulo, 'Parque Estadual das Fontes do Ipiranga' (23°38'31.3"S 46°37'29.2"W)	FIFUNGI-273
CCIBt4784	*Favolus rugulosus*	MPD240	Brazil, São Paulo, Cananeia, 'Parque Estadual da Ilha do Cardoso', 'Núcleo Marujá', Tapera trail	SP528743

Culture media preparation

Potato Dextrose Agar (PDA, Kasvi, Spain) was prepared following the product recommendation, with a concentration of 39 g/L; Malt Yeast Peptone Agar (MYPA) was prepared using 20 g/L of Malt Extract (Kasvi, Spain), 2 g/L of Yeast Extract (Kasvi, Spain), 1,5 g/L of Soy Peptone (Kasvi, Spain), and 15 g/L of Ultra-pure Agar powder (Himedia, India); Yeast Glucose Agar (YGA) was prepared using 5 g/L of Yeast Extract, 20 g/L of Glucose, and 12 g/L of Ultra-pure Agar powder; the Soy Agar (SOY) recipe was adapted from Thawthong et al. (2014): 50 g of commercial organic raw soybeans [*Glycine max* (L.) Merr. – Coopernatural, Brazil] were soaked overnight in 250 mL of filtered water, and then boiled for 30 minutes; the boiled soybeans were then grounded, using a mortar and pestle, and filtered through a clean cheesecloth, squeezing out most of the liquid; 20 g of Ultra-pure Agar powder was added to the filtrate and the volume was adjusted to 1 L by adding distilled water. All media were autoclaved at 15 psi and 121 ºC for 15 minutes. A total of 30 mL of each medium was poured into 90 mm plastic Petri dishes under a laminar flow hood.

Experimental design

For the evaluation of the effect of the temperature and culture media under the mycelial growth velocity and biomass production of each strain, separate experiments were conducted for each species of *Favolus*. Four temperatures (20 ºC, 25 ºC, 30 ºC, and 35 ºC) and four solid culture media (MYPA, PDA, SOY, and YGA) were tested. For each treatment, 10 replicates of each strain were used.

For the experiments, mycelium plugs of 10 mm diam. were used as inoculum and transferred to the center of the Petri dishes containing each medium, which were then incubated

at the four different temperatures. The mycelium growth was monitored daily, and the experiment was stopped for all the treatments when one of the replicates of each species totally colonized the plate. The mycelium growth was then measured by the colony diameter of each replicate. For the measurement of the mycelium dry weight, the medium of each replicate was removed from the Petri dish and transferred to 300 mL beakers with water and microwaved for 2 minutes or until the medium was totally melted; the mycelium was then washed with hot water and filtered on a paper filter under vacuum and the filtered mycelia were dehydrated at 50 ºC until constant weight, when the weights of each replicate were then annotated.

Statistical and data analyses

Data analyses and graphic construction were conducted using the R software (R Core Team, 2023) under the RStudio environment (RStudio Team, 2023) with the "ggplot2" package (Wickham, 2016). Statistical analyses of our data were performed with the "car" package (Fox & Weisberg, 2019). Assumptions of normality and homogeneity of variances from our data were ascertained by the Shapiro-Wilk and Levene tests, respectively (Zar, 1999). Non-homoscedastic or non-normal dependent variables were transformed by the Box-Cox method using the exact calculated lambda value (Box & Cox, 1964) or by other transformations such as logarithmic or square-root transformations. For *F. brunneofibrillosus* and *F. rugulosus*, experiments with only one strain per species, significancy of the difference between the means of each treatment and their interaction were evaluated by a two-way ANOVA test, and the different combinations were compared with the Tukey HSD test. For the *F. brasiliensis* experiment with two strains, a three-way ANOVA test was conducted, followed by Tukey HSD test. Considering the equivalent interpretation of our data with transformed and non-transformed variables, graphics were plotted with the non-transformed response variables for ease of interpretation.

Results

For *F. brasiliensis*, one of the replicates of the wild strain CCIBt4770 growing on MYPA at 25 ºC was the first to fully colonize the plate in 14 days and no major differences in the density of the mycelium mats were noted for the replicates growing on the different media (Fig. 1). The wild strains CCIBt4768 and CCIBt4770, however, were not statistically different ($p > 0.05$) when growing on MYPA at 25 ºC and grew equally well in diameter. Additionally, the wild strain CCIBt4770 growing on either SOY and YGA at 25 °C and MYPA at 30 °C were also not significantly different from the combination of MYPA at 25 °C. The second-best factor combinations ($p \leq 0.05$) for the mycelium growth were observed on either MYPA, PDA or YGA at 20 °C, PDA at 25 ºC or SOY and YGA at 30 ºC for CCIBt4770, and on MYPA at 20 ºC for CCIBt4768. Concerning the biomass production, the wild strain CCIBt4770 achieved the best mycelium dry weight ($p \leq 0.05$) compared to the strain CCIBt4768 when growing on either MYPA (224.98 mg ± 43.90 mg) or YGA (276.16 mg ± 55.75 mg) at 25 ºC. The second-best factor combinations ($p \leq 0.05$) for the biomass production were noted on either MYPA at 20 ºC (102.28 mg ± 20.41 mg), PDA (89.88 mg ± 17.30 mg) or YGA (113.7 mg ± 13.35 mg) at 25 ºC, and YGA at 30 ºC (91.04 mg ± 17.99 mg) for CCIBt4768 strain, whilst for CCIBt4770 the second-best combination ($p \leq 0.05$) was noted on MYPA at 30 ºC (169.97 mg ± 32.94 mg).

Despite the equivalent growth in diameter of the *F. brasiliensis* wild strains on the best factor combination (MYPA at 25 ºC), the strain CCIBt4770 was able to produce a significant higher biomass when growing at the best factor combinations when compared to the strain CCIBt4768, while also outperforming the CCIBt4768 wild strain on most combinations. Based on these findings, we recommend that the wild strain CCIBt4770 be prioritized for subsequent domestication studies. Notably, the most favorable growth conditions related to this strain involve the utilization of either MYPA or YGA media at a temperature of 25 °C.

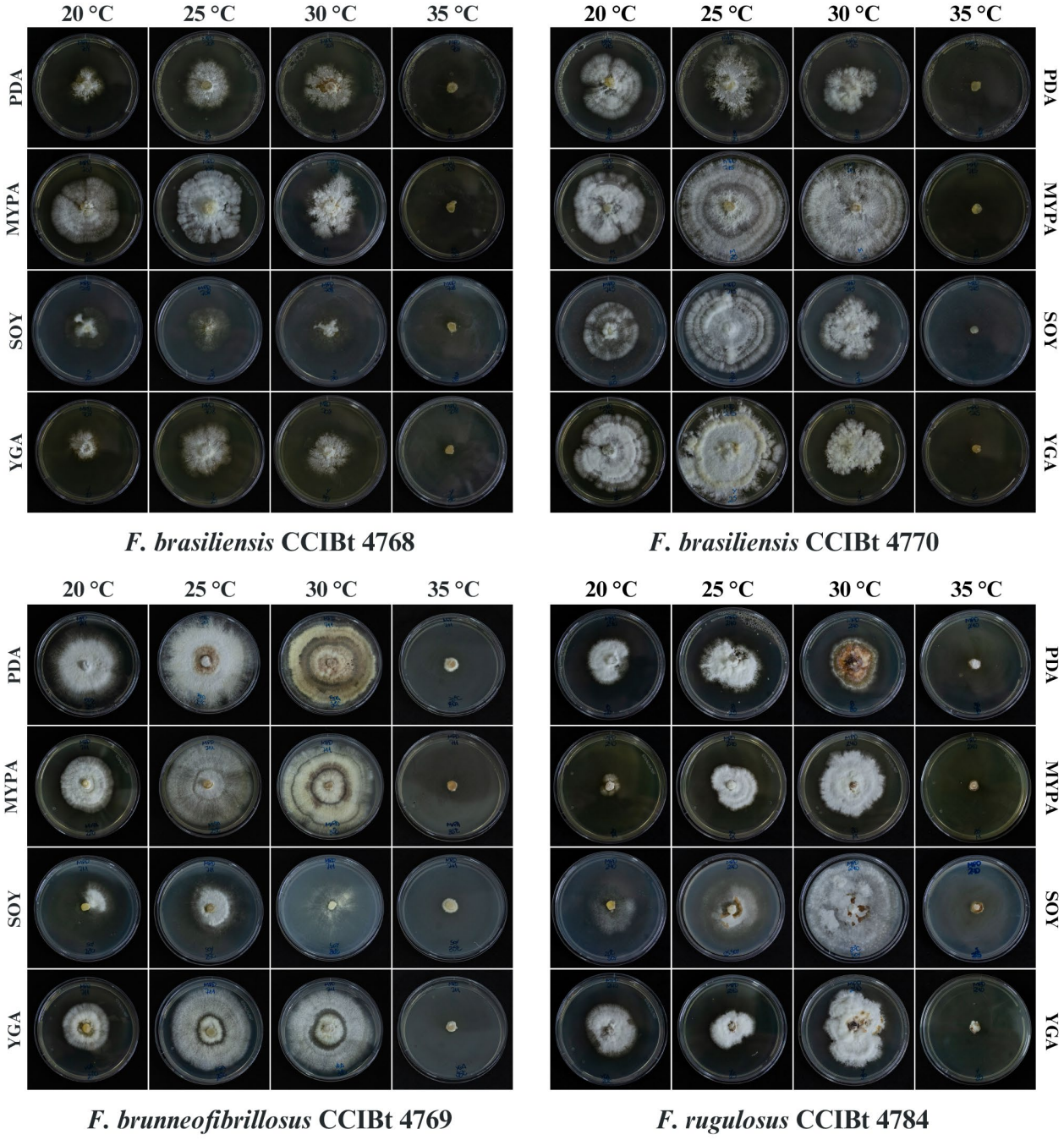

Fig. 1 Culture growth and morphology of the *Favolus* spp. strains at the end of the experiment.

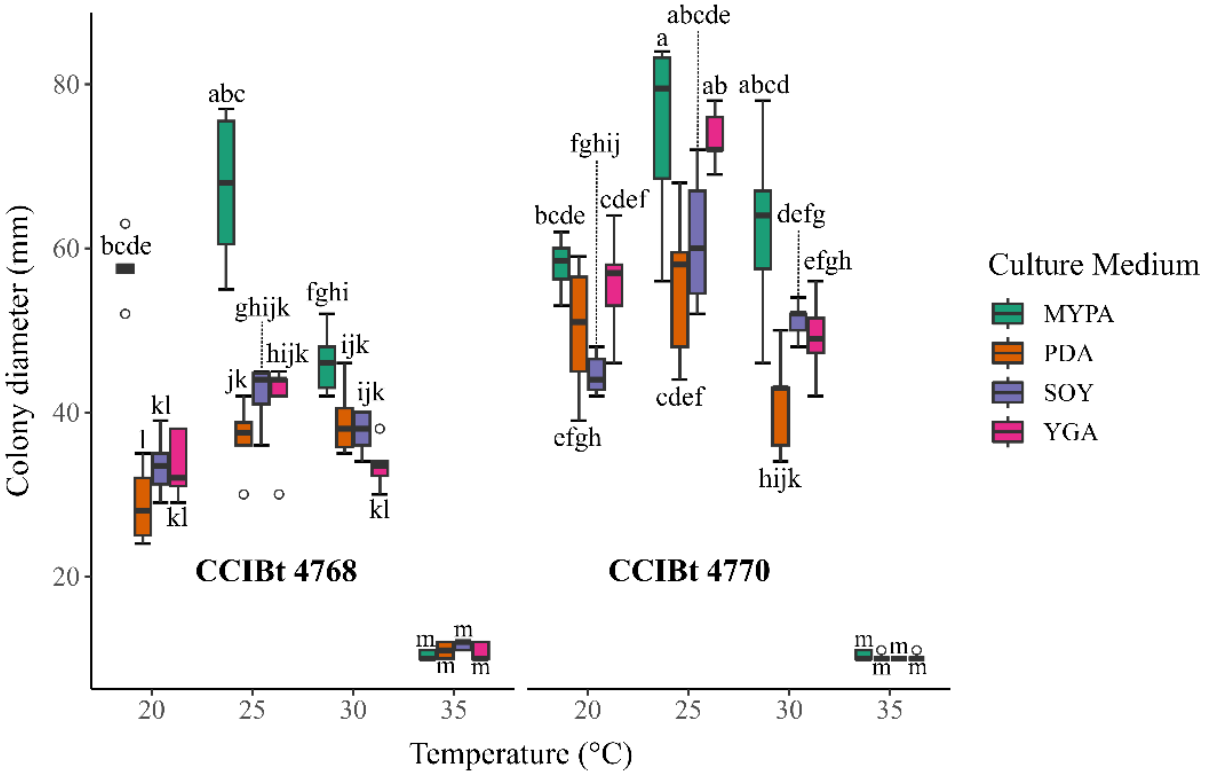

Fig. 2 Effect of different temperatures and solid culture media on the colony diameter of two Brazilian wild strains of *F. brasiliensis* (CCIBt4768 and CCIBt4770) on the 14th day. White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$ (based on the logarithmic transformed data of the colony diameter).

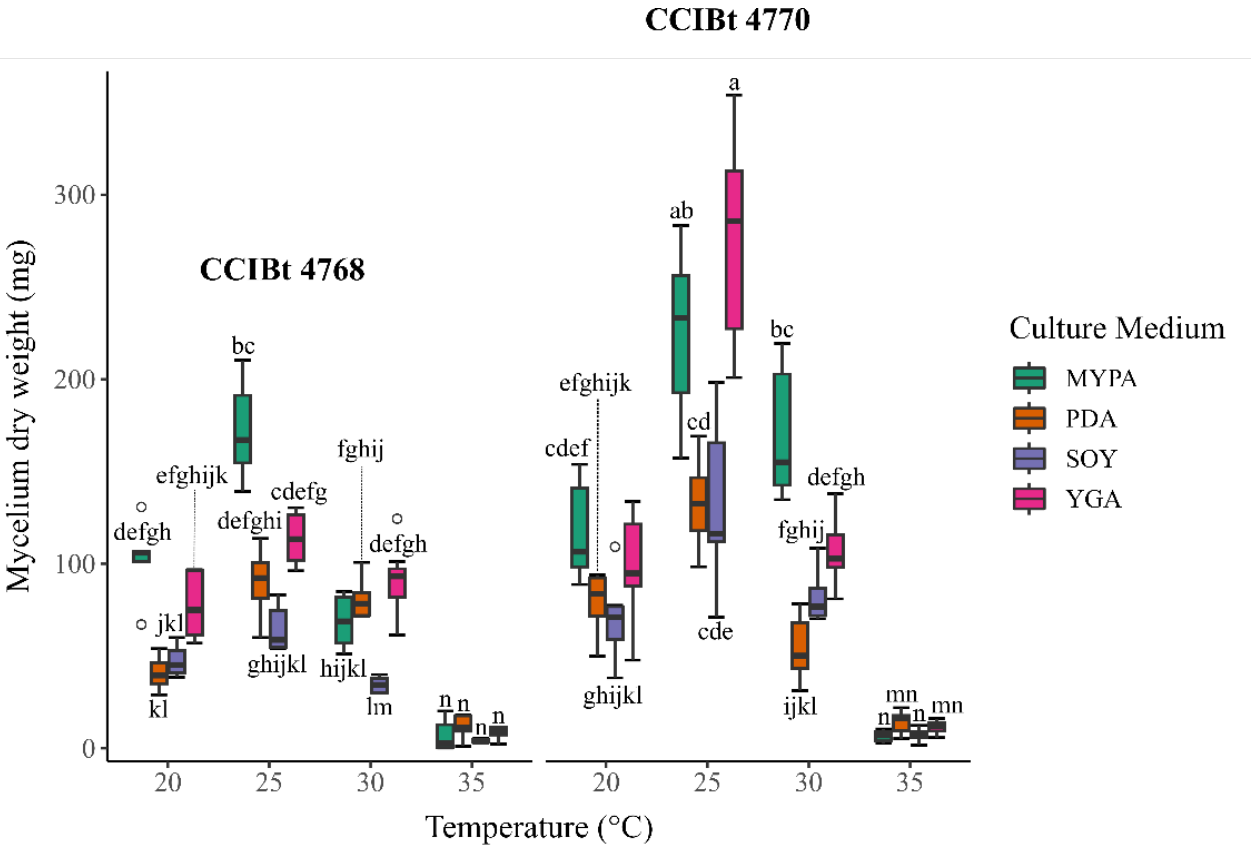

Fig. 3 Effect of different temperatures and solid culture media on the mycelium dry weight of two Brazilian wild strains of *F. brasiliensis* (CCIBt4768 and CCIBt4770) on the 14th day.

White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$ (based on the Box-Cox transformed data of the mycelium dry weight).

For *F. brunneofibrillosus*, the wild strain CCIBt4769 was able to achieve the best ($p \leq 0.05$) mycelium growth in diameter on either MYPA and PDA at 30 ºC and on PDA at 25 ºC, fully colonizing the plate on PDA at 30 ºC in 12 days. The second-best factor combination ($p \leq 0.05$) for the mycelium growth was found with YGA at 30 °C. For the biomass production, the best factor combinations ($p \leq 0.05$) were either PDA (350.44 mg ± 25.71 mg) and MYPA (312.12 mg ± 39.45 mg) at 30 ºC and PDA at 25 ºC (324.37 mg ± 18.71 mg), followed ($p \leq 0.05$) by either YGA at 30 ºC (175.43 mg ± 16.04 mg), MYPA (183.43 mg ± 35.74 mg) and YGA (143.50 mg ± 29.07 mg) at 25 ºC, and PDA at 20 ºC (147 mg ± 17.78 mg). Even though the replicates growing on SOY were able to colonize the plates well in diameter when incubated at 25 ºC and 30 ºC, the culture morphology seemed the most affected, with a very thin and scant mycelium mat (Fig. 1), and the biomass production was very low. Moreover, cultures of *F. brunneofibrillosus* growing on PDA had the densest mycelium mat, which correlates to the better biomass production of the strain on this medium. MYPA and PDA at either 25 ºC or 30 ºC are the combinations related to the best mycelium growth when compared to the temperatures and media tested here for the wild strain CCIBt4769 and should be considered for further domestication studies of *F. brunneofibrillosus*.

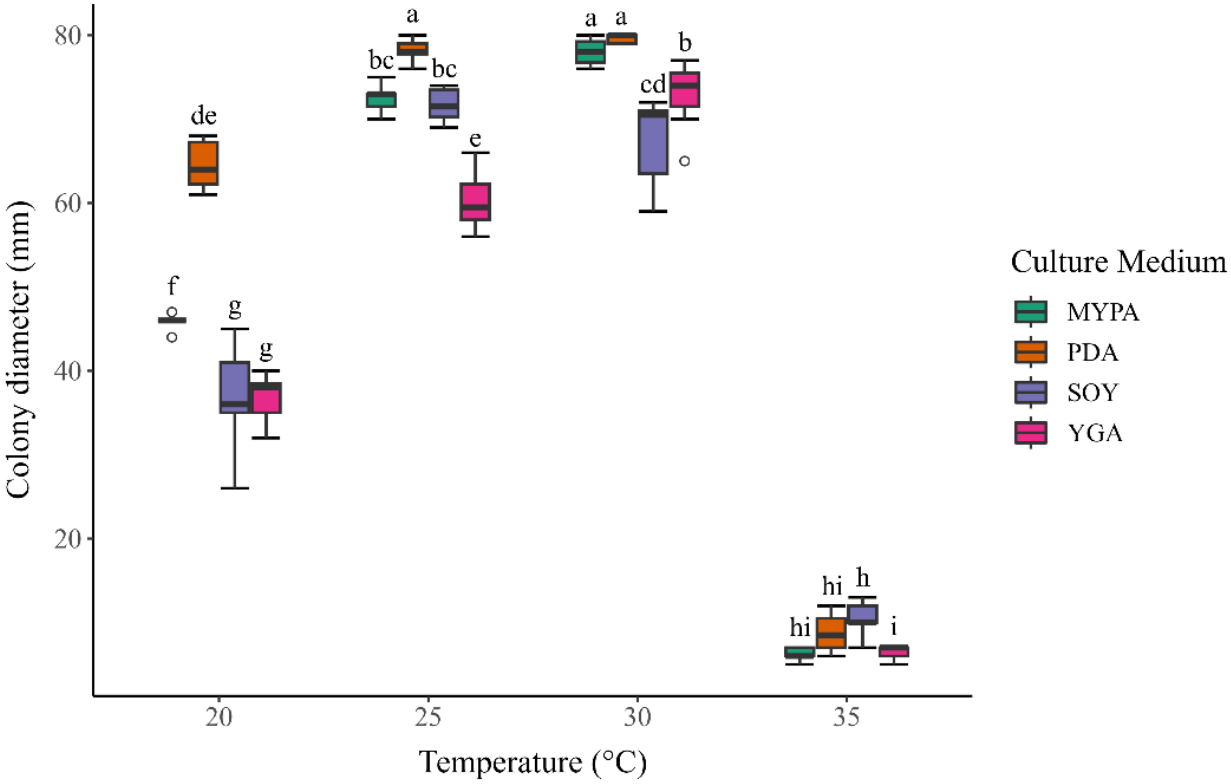

Fig. 4 Effect of different temperatures and solid culture media on the colony diameter of the wild strain CCIBt4769 of *F. brunneofibrillosus* on the 12th day. White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$.

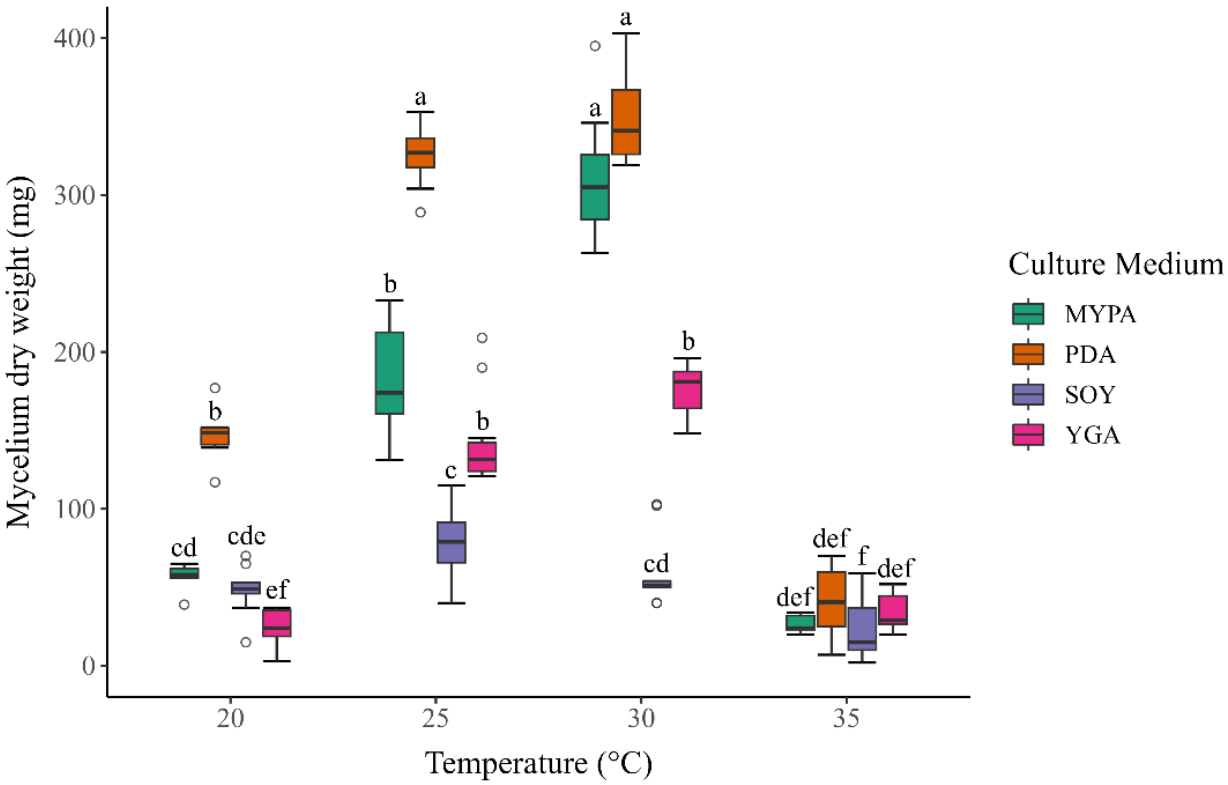

Fig. 5 Effect of different temperatures and solid culture media on the colony dry weight of the wild strain CCIBt4769 of *F. brunneofibrillosus* on the 12th day. White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$ (based on the square-root transformed data of the mycelium dry weight).

The wild strain CCIBt4784 of *F. rugulosus* achieved the best mycelium growth in diameter on SOY at 30 ºC by far, fully colonizing the plate in 10 days. The second-best factor combinations ($p \leq 0.05$) for the mycelium growth were either YGA and MYPA at 30 ºC and SOY at 25 ºC ($p > 0.05$). The best biomass production was achieved on SOY at 30 ºC (183.66 mg ± 46.53 mg) or SOY at 25 °C (139.2 mg ± 20.67 mg) when compared to the other combinations ($p \leq 0.05$), with the second-best factor combinations ($p \leq 0.05$) being either PDA (124.03 mg ± 32.84 mg), MYPA (123.6 mg ± 25.89 mg), and YGA (115.2 mg ± 33.95 mg) at 30 ºC. Replicates growing on MYPA and YGA media produced the thickest and densest mycelium mat, while those growing on the SOY medium had a scanter mycelium mat, although denser than the *F. brunneofibrillosus* replicates growing on SOY (Fig. 1). Despite this, the way faster mycelium growth of the replicates on SOY at 30 ºC compared to those growing on MYPA and YGA favored a higher biomass production on the first parameter combination when compared to the other combinations with thicker mycelium mats but with slower growth. Considering our findings, we advise the consideration of the use of the SOY medium and incubation at 30 ºC for further domestication studies of *F. rugulosus* strains.

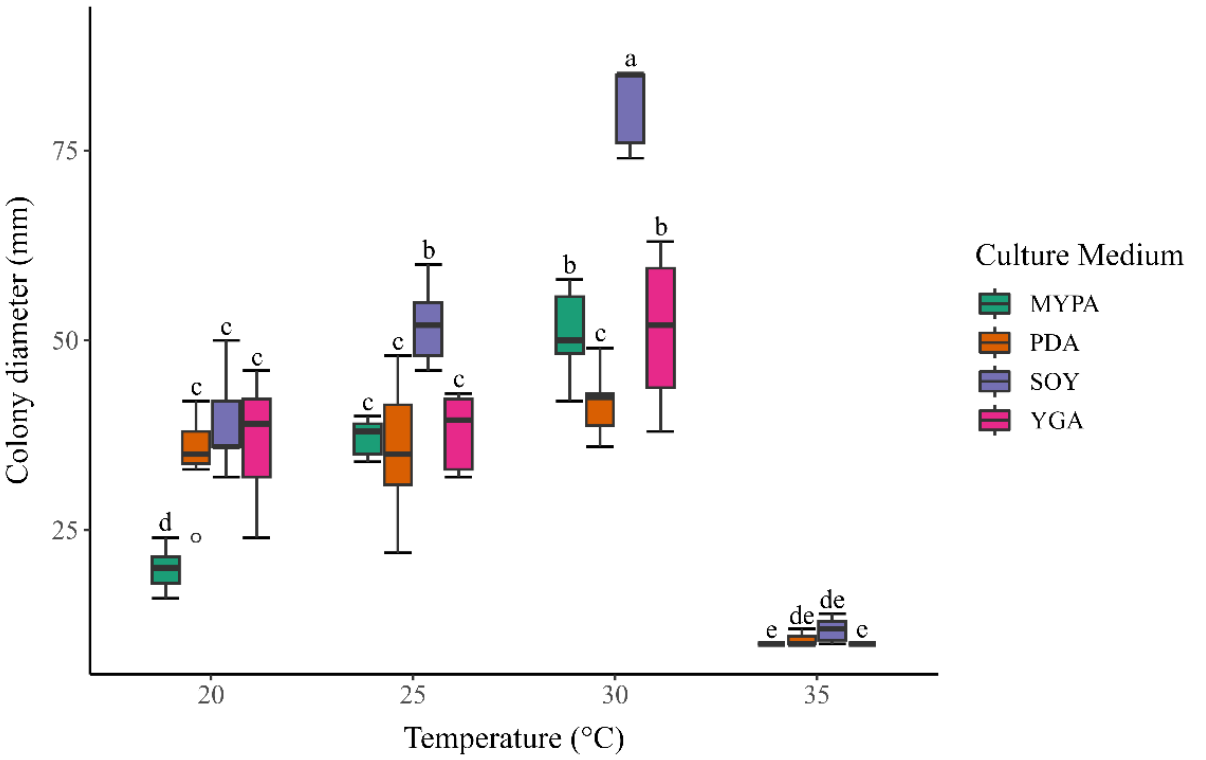

Fig. 6 Effect of different temperatures and solid culture media on the colony diameter of the wild strain CCIBt4784 of *F. rugulosus* on the 10th day. White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$.

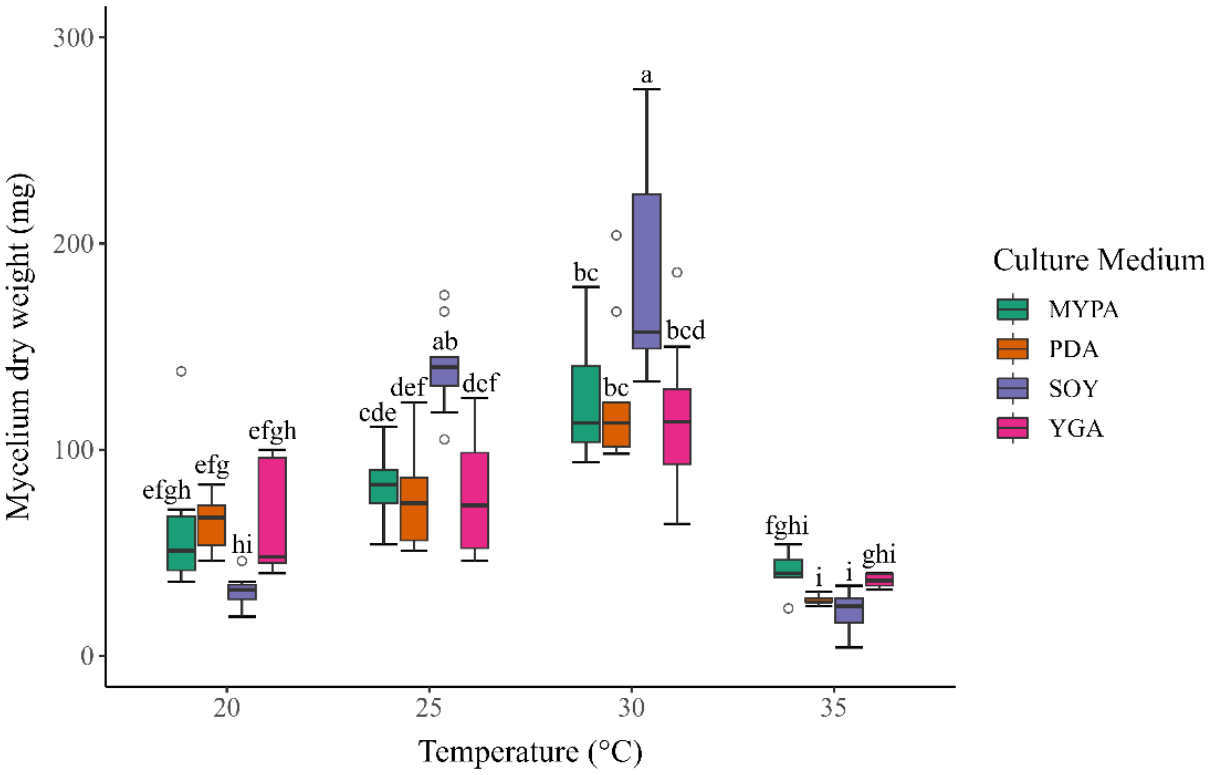

Fig. 7 Effect of different temperatures and solid culture media on the colony dry weight of the *F. rugulosus* CCIBt4784 strain on the 10th day. White dots represent outliers. Values not sharing common letters are significantly different with $p \leq 0.05$ (based on the Box-Cox transformed data of the mycelium dry weight).

Discussion

Our study showed that the selected wild strains of *F. brasiliensis*, *F. brunneofibrillosus*, and *F. rugulosus* were able to grow well at a temperature range from 20 ºC to 30 ºC, with the temperatures related with the best mycelium growth and biomass production being either 25 or 30 ºC. Additionally, all replicates incubated at 35 ºC were not able to grow or grew very poorly. Mswaka & Magan (1999) when studying the growth of wood decaying fungi on different temperatures classified them into three different groups: (1) low-temperature group with an optimal temperature range of 25–30 ºC, and with no growth above 37 ºC; (2) intermediate group with optimal temperature range of 30–37 ºC and with no growth at 45 ºC; and (3) the high-temperature group with optimal temperature range 37–40 ºC, with growth ceasing at 55 ºC. The optimal temperature range of 25–30 ºC and the poor or absence of growth at 35 °C of the *Favolus* spp. strains tested here would classify them as being of the low-temperature group. Our findings are similar to a previous domestication study of *Favolus* by Omarini et al. (2009) that reported the best mycelium growth for two strains of *Favolus* sp. [as *Polyporus tenuiculus* (P. Beauv.) Fr.] from the South region of Brazil at 30 °C. The better mycelium growth at 25–30 °C of our strains is also similar to domestication studies of other wild edible mushrooms from tropical regions, such as *Pleurotus albidus* (Berk.) Pegler, *P. djamor* (Rumph. ex Fr.) Boedijn, and *P. pulmonarius* (Fr.) Quél. by Lechner & Albertó (2011), *Lentinus connatus* Berk., *L. roseus* Karun., K.D. Hyde & Zhu L. Yang, and *Pleurotus giganteus* (Berk.) Karun. & K.D. Hyde by Klomglung et al. (2014), *L. squarrosulus* Mont. by De Leon et al. (2013), and *L. tigrinus* (Bull.) Fr. by Dulay et al. (2012), which report optimal temperatures for mycelium growth ranging from 25 ºC to 30 ºC. Some tropical strains are even reported to grow at temperatures higher than 30 °C and even up to 45 °C, such as *Panus neostrigosus* Drechsler-Santos & Wartchow (Vargas-Isla & Ishikawa, 2008, as *Lentinus strigosus* Fr.) and *L. squarrosulus* (Gbolagade et al. 2006). Temperature is one of the most important and critical factors affecting mycelium growth in mushroom cultivation. Elucidation of the optimal temperature is very important for an efficient mycelium growth with adequate biomass, production of metabolic products, and formation of basidiomata (Chang & Miles, 2004). Increasing the temperature generally accelerates enzymatic activity and improve the metabolic efficiency of mushrooms, but a high enough temperature can denature or inactivate enzymes, which, in turn, can stunt mycelium growth (Chang & Miles, 2004). This can explain the poor or absence of growth of our strains at 35 °C, which could be above the maximum temperature supported by these *Favolus* spp. strains.

The results from our experiments also showed a differential growth and biomass production of the *Favolus* spp. strains growing on the different solid culture media. Nutrients such as carbon and nitrogen sources, minerals (such as phosphorus, potassium, and magnesium) and vitamins (such as thiamin and biotin) are essential for a healthy mycelium growth, and the composition and proportion of these nutrients in different culture media can also affect the efficiency of mycelium growth and biomass production of mushroom strains (Chang & Miles, 2004). Considering the high availability and diversity of raw materials, such as grains and legumes in Brazil, we opted to test the effect of a solid culture media made with soybeans on the mycelium growth of our strains. Soybeans are one of the most nutritional beans available at markets in Brazil, providing one of the most complete vegetable protein sources and also being a source of carbohydrates, vitamins, and minerals (USDA, Agricultural Research Service, FoodData Central, 2019). Our results evidenced the best mycelium growth and dry mycelium

weight of the wild strain CCIBt4784 of *F. rugulosus* on the SOY medium. Despite this, the other studied strains, while also being able to grow on the SOY medium, sometimes presented an abnormal culture morphology and a very scant mycelium, especially for the wild strain CCIBt4769 of *F. brunneofibrillosus*. Our findings from the *F. brasiliensis* and *F. brunneofibrillosus* experiments seem to differ from those of Klomklung et al. (2014) that reported a way better mycelium growth with a high mycelium density of strains of *Lentinus* and *Pleurotus* from Thailand growing on solid culture media made with various grains and legumes, including soybean when compared to PDA and MEA.

Still regarding the differential growth on different solid culture media, a previous cultivation study of *F. rugulosus* by Sanchez-Ocampo et al. (2022), found that a Colombian strain was able to achieve the best mycelium growth in area at 23 ºC on YGA solid medium supplemented with the antibiotic oxytetracycline (58,38 cm^2), followed by Malt Extract Agar (33,76 cm^2) and PDA (20,99 cm^2). They also noticed a denser and a more cottony mycelium mat on the replicates growing on YGA, and a less dense and irregular mycelium mat on PDA. These findings are similar to ours in relation to the mycelium morphology of our replicates growing at YGA. Additionally, we also noticed irregular growth on some replicates growing on PDA, which presented a scant or abnormal mycelium mat, even though some replicates presented a regular morphology (Fig. 1). Veloso et al. (2023) also studied the mycelial growth of a wild strain of *F. rugulosus* from Paraguay on different solid culture media and found a more efficient growth of the strain on Malt Extract Agar (25,1 mm/day), followed by PDA (24,5 mm/day) and Sabouraud (22,4 mm/day).

Finally, our study highlights the presence of a statistically significant interaction effect between each of the studied factors, such as the temperature and culture medium, or even the broader combination of temperature, culture media and strain on the mycelium growth. This interaction between temperature and culture media, as well as the additional layer introduced by the addition of fungal strains, sheds light on the complexity inherent in studying fungal growth dynamics. One possible underlying explanation for the interaction effect we have observed lies in the metabolic dynamics of the fungal strains employed. It is plausible that these strains exhibit distinct metabolic efficiencies, influencing their ability to effectively utilize and convert the nutrients in the different culture media when subjected to different incubation temperatures. Nevertheless, it is important to acknowledge the complexity that accompanies the comprehensive statistical analyses for comparison of the various factor combinations, which is even further magnified when considering the inclusion of multiple fungal strains. This complexity has been also previously highlighted by Kaufman et al. (1963) when studied the interaction effect of soil, temperature, and culture media for selection of a single temperature or medium for the isolation of soil fungi. As the number of factors widens, it becomes increasingly vital to strike a balance between experimental complexity and data interpretability. Even though we were able to detect significant combinations related to better mycelium growth responses, our study also calls attention to the potential challenges associated with these multifactorial analyses, and we hope it serves as a foundation for future research endeavors seeking to unravel the intricate interaction of growth factors in fungal cultural studies.

Considering the optimal temperature range of the studied *Favolus* spp. strains to be between 25 °C and 30 °C and the possibility of growth on cheap and readily available agro-industrial residues, we believe this study could help further domestication studies of edible *Favolus* species that have potential for the commercial cultivation as food and expansion of the still restricted edible mushroom market in Brazil with local species.

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CONSIDERAÇÕES FINAIS

Os resultados deste trabalho contribuíram com o conhecimento acerca da diversidade do gênero *Favolus* no Brasil e sua ocorrência e distribuição em áreas de Mata Atlântica dos estados de São Paulo e do Rio de Janeiro.

Neste trabalho, foram identificados 86 espécimes de *Favolus,* que correspondem a cinco táxons aqui descritos, sendo dois táxons propostos como espécies novas. Desses 86 espécimes, 71 espécimes foram identificados como *F. brasiliensis,* cinco espécimes como *F. brunneofibrillosus,* um espécime como *Favolus glaucovelutinus*, oito espécimes como *F. rugulosus* e um espécime como *Favolus radiatifibrillosus.* O número de espécimes coletados de *F. brasiliensis* reflete a alta frequência dessa espécie durante as saídas de campo realizadas ao longo do projeto, sendo encontrada em todas as saídas e comumente encontrada também em coletas esporádicas. A partir das análises filogenéticas moleculares, foi também possível elucidar a ampla distribuição dessa espécie pelo continente americano, ocorrendo desde a Argentina até a região ao sul dos Estados Unidos.

Além disso, foram geradas neste trabalho 87 novas sequências de espécimes de *Favolus,* sendo 35 sequências de ITS, 25 de nucLSU, 19 de *TEF1* e oito de *RPB1.* Infelizmente, não tivemos sucesso no sequenciamento do gene *RPB1* para os espécimes das duas espécies novas descritas e tampouco para *Favolus radiatifibrillosus*, apesar do sucesso na amplificação e purificação dos produtos da PCR. Apesar disso, foram geradas as primeiras sequências de DNA (ITS, nucLSU e *TEF1*) para um espécime coletado, assim como para dois parátipos, de *F. radiatifibrillosus*, uma espécie recentemente descrita para o Brasil. A geração dessas novas sequências também possibilitou um melhor entendimento das relações filogenéticas entre as espécies neotropicais de *Favolus* e, especialmente, para *F. brasiliensis*, um táxon considerado anteriormente como um complexo de espécies, mas que, no entanto, em nossa reconstrução com uma maior amostragem e com mais marcadores, foi recuperado como um único clado.

Os estudos morfológicos realizados para identificação e descrição dos materiais estudados permitiram o reconhecimento da presença de caracteres mais informativos para a identificação dos espécimes de *Favolus*, como a forma e organização dos poros no himenóforo, as dimensões dos basidiósporos e, principalmente, a estrutura e organização da superfície pilear. A superfície do píleo das espécies estudadas pode ser glabra ou enrugada, com padrões de linhas radiais, ou com estruturas fibrilosas organizadas radialmente ou de forma reticulada. Além disso, diferentes graus de pubescência são observados, podendo ser irregularmente ou densamente pubescente a velutina e com pelos de tamanho variado. Contudo, é bastante notável a variabilidade de certos caracteres macromorfológicos para algumas das espécies, o que pode dificultar a identificação de espécimes em campo. Consequentemente, salientamos a importância, sempre que possível, de dados moleculares, fisiológicos ou ecológicos complementares à morfologia para a identificação e a realização de novos estudos taxonômicos do gênero.

Em relação ao estudo dos fatores relacionados ao crescimento micelial *in vitro* dos isolados selecionados de *Favolus*, é pertinente ressaltar a aparente dificuldade do isolamento de espécimes de *Favolus* a partir de fragmentos do contexto. Apesar dos esforços para o isolamento de grande parte dos espécimes coletados em campo, apenas quatro isolados se mostraram viáveis para a realização dos experimentos. As tentativas de isolamento de espécies de *Favolus* tanto em campo quanto em cabine de fluxo laminar e utilizando as boas práticas de

assepsia antes e duramente o isolamento, geralmente resultavam em ausência de crescimento micelial dos fragmentos do contexto inoculados, ou então uma rápida contaminação por bactérias e outros fungos filamentosos, como *Apiospora* sp., *Bjerkandera* sp., *Clonostachys* sp., *Neopestalotiopsis* sp., *Schizophyllum commune*, *Trichoderma* sp. e *Xeromyces* sp., identificados por meio do sequenciamento de amostras de DNA extraídos das culturas. Outros fatores que também podem estar vinculadas ao número reduzido dos isolados de *Favolus* nesse estudo estão relacionadas com as dificuldades da pandemia da COVID19, como a dificuldade de acesso ao instituto para manutenção das culturas, e problemas estruturais, como o mal funcionamento das geladeiras onde estavam sendo mantidas algumas culturas preservadas em Castellani e que levou ao congelamento das amostras. Apesar disso, com a análise dos isolados que permaneceram viáveis, nossos resultados puderam evidenciar um crescimento ótimo para as espécies avaliadas (*F. brasiliensis*, *F. brunneofibrillosus* e *F. rugulosus*) quando incubadas a 25 ou 30 ºC, o que é bastante promissor quando considerado o potencial como alimento para o cultivo comercial em regiões tropicais dessas espécies de *Favolus*. Ademais, os isolados foram capazes de crescer em todos os meios de cultura testados, apesar de um crescimento irregular de algumas réplicas da mesma espécie, em meios como o Ágar Soja ou Batata Dextrose Ágar. A elucidação das melhores combinações de meios de cultura e temperaturas de incubação para os isolados de *Favolus* selecionados poderão orientar futuros estudos de domesticação de espécies promissoras do gênero, incluindo a avaliação da eficiência de colonização e conversão de nutrientes de diferentes substratos, além dos fatores relacionados à indução da formação dos basidiomas.