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MACROFUNGOS AGARICALES EM ÁREAS DE MANEJO FLORESTAL NA AMAZÔNIA CENTRAL

DIRCE LEIMI KOMURA

Manaus, Amazonas
Janeiro, 2016

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Orientador: Dr. CHARLES EUGENE ZARTMAN
Co-orientador: Dr. JEAN-MARC MONCALVO

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RELAÇÃO DA BANCA EXAMINADORA DA AULA DE QUALIFICAÇÃO:

Prof. Dr. Antônio Carlos Webber (Universidade Federal do Amazonas- UFAM)

Dr. Michael John Gilbert Hopkins (Instituto Nacional de Pesquisas da Amazônia- INPA)

Dr. José Luis Camargo (Instituto Nacional de Pesquisas da Amazônia- INPA)

Dr. Sérgio Pérez Gorjón (CIEFAP- ARGENTINA)

Dr. Jean-Marc Moncalvo (Royal Ontario Museum- ROM and University of Toronto- Canadá)

RELAÇÃO DA BANCA EXAMINADORA DA DEFESA PÚBLICA:

Dr. Felipe Wartchow (Universidade Federal da Paraíba- UFPB)

Dra. Ruby Vargas-Isla (Instituto Nacional de Pesquisas da Amazônia- INPA)

Dra. Cristina Sayuri Maki (Universidade Federal do Amazonas- UFAM)

Dr. Leandro Rafael de Assis (Instituto Nacional de Pesquisas da Amazônia- INPA/ MAX PLANCK)

Dr. Mario Henrique Terra-Araújo (Instituto Nacional de Pesquisas da Amazônia- INPA)

Suplentes:

Prof. Dr. Antônio Carlos Webber (Universidade Federal do Amazonas- UFAM)

Dr. Alberto Vicentini (Instituto Nacional de Pesquisas da Amazônia- INPA)

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Sinopse:

Estudos sobre a diversidade de macrofungos Agaricales em áreas de manejo florestal na Amazônia Central. Aspectos ecológicos e taxônomicos foram avaliados.

Palavras-chave: *Marasmius* spp., *Tetrapyrgos* spp., taxonomia, ecologia, floresta secundária, floresta primária.

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"Quando descobriu que suas crenças, há muito estimadas, não concordavam com as observações mais precisas, ele (Johannes Kepler) aceitou os fatos desconfortáveis. Ele preferiu a dura verdade às suas ilusões mais caras. Isto é o coração da Ciência!" (Cosmos- A Harmonia dos Mundos - episódio 3- Carl Sagan)

RESUMO

Esta tese refere-se ao estudo de macrofungos Agaricales em floresta de terra firme na Estação Experimental de Manejo Florestal do INPA (ZF-2). Durante o período de dois anos, 669 basidiomas (corpos de frutificação) foram coletados nos oito transectos (5 × 50 m cada) localizados em áreas de floresta primária e secundária. Um total de 287 espécies/morfotipos foram identificados durante o estudo. A composição de macrofungos foi diferente entre floresta primária e secundária, diferindo também de acordo com a estação e o ano da coleta. A detecção de macrofungos foi muito baixa na estação seca, principalmente na floresta secundária. Observou-se que, em relação aos substratos (troncos, galhos, folhas e solo), a floresta primária apresentou maior índice de riqueza e diversidade para "solo". Por outro lado, no substrato "folhas", estes índices foram maiores para floresta secundária. Os fungos marasmioides e gymnopoides apresentaram um número expressivo de espécies/morfotipos. Em virtude disso, a descrição morfológica usando caracteres macro e microscópicos aliada aos dados moleculares com sequenciamento da região ITS (*internal trancribed spacer*) foi realizada para estas espécies. Esta etapa do trabalho resultou na descrição de seis espécies novas de *Tetrapyrgos* (nomes provisórios: *T. albonigripes*, *T. brevipileocystidiata*, *T. brunneilucida*, *T. cystidiacrassa*, *T. pileobrunnea*, e *T. pseudonigripes*) e no registro de nova ocorrência para a Amazônia de *T. longicystidiata*. *Marasmius calvocystidiatus* (nome provisório) é descrito como nova espécie, cuja espécie irmã, *Marasmius horridulus*, é recoletada pela primeira vez após sua descrição. Nove espécies de *Marasmius* que apresentam a formação de rizomorfos foram descritas, dentre estas, *M. cupressiformis*, *M. populiformis* e *M. microdendron*. Além disso, foram também descritos 37 táxons de *Marasmius* da seção *Marasmius*, dos quais 21 em nível de espécie. Em relação às espécies compreendidas em Omphalotaceae (*Gymnopus*, *Marasmiellus* e *Rhodocollybia*), restringiu-se à apresentação de dados moleculares e sinopses. De modo geral, o presente trabalho abre veredas que, espera-se que, possam auxiliar novos estudos interessados na compreensão de macrofungos da Amazônia.

Palavras-chave: Agaricales, ecologia, floresta secundária, floresta primária, *Marasmiellus* spp., *Marasmius* spp., taxonomia, *Tetrapyrgos* spp.

ABSTRACT

This thesis is an ecological and taxonomic account of Agaricales macrofungi in a terra firme forest at the Estação Experimental de Manejo Florestal do INPA (ZF-2). Over a two year period 669 basidiomes (fruiting bodies) were collected in eight transects (5 × 50 m each), located in primary and secondary forests. A total of 290 species/ mophospecies were identified during the study. The macrofungal composition was different among primary and secondary forests, and this difference was also observed among seasons and collection year. In the dry season, the secondary forest presented the lowest macrofungal richness and abundance when compared with primary forest. In relation to substrate type (trunk, branches, leaves and soil), basidiome richness of leaf litter guilds was higher in secondary forest plots, whereas, in contrast, soil guild richness was greater in primary forest. Marasmioid and gymnopoid fungi were the most representative group in this study, and taxonomic descriptions using macro and microscopic characters complemented with molecular data, ITS (internal trancribed spacer), were carried out in these groups. The taxonomic stage resulted in description of six new species for *Tetrapyrgos* (provisional names: *T. albonigripes*, *T. brevipileocystidiata*, *T. brunneolucida*, *T. cystidiacrassa*, *T. pileobrunnea*, and *T. pseudonigripes*) and *T. longicystidiata* as newly registered new recording to Amazonia. *Marasmius calvocystidiatus* (provisional name) is described as new species and its sister species *M. horridulus*, which just its type specimen was known is recollected. Nine species with rhizomorph are described, among them, *M. cupressiformis*, *M. populiformis* e *M. microdendron*. In addition, 37 taxa of *Marasmius* are presented, from which 21 are describe at the species level. For the species included in Omphalotaceae (*Gymnopus*, *Marasmiellus* and *Rhodocollybia*), we restrict to the presentation of molecular data and synopses about them. Overall, this work intends to open up paths for future studies of macrofungal taxonomy and ecology in the Amazon basin.

Palavras-chave: Agaricales, ecologia, floresta secundária, floresta primária, *Marasmiellus* spp., *Marasmius* spp., taxonomia, *Tetrapyrgos* spp.

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APRESENTAÇÃO

Esta tese apresenta os resultados do projeto "Macrofungos Agaricales em Áreas de Manejo Florestal na Amazônia Central". A área principal de estudo foi a Estação Experimental de Manejo Florestal ZF-2, localizada a aproximadamente 80 km N de Manaus, Amazonas. Nesta área foram montados oito transectos em florestas de terra firme primária e secundária, os quais foram monitorados ao longo de dois anos para o estudo ecológico. Além desta área, para os estudos taxonômicos foram incorporados ao estudo, materiais provenientes de outras áreas da Amazônia, a saber: Rio Cuieiras, Reserva Ducke, campus do INPA, Reserva de Desenvolvimento Sustentável do Tupé, no Amazonas; Parque Nacional do Viruá, em Roraima e Parque Nacional do Tapajós, no Pará. Com isso, objetivou-se ampliar o conhecimento de macrofungos Agaricales no que tange aos seus aspectos ecológicos e taxonômicos. Neste último aspecto, incluiu-se o caracter molecular com o sequenciamento da região ITS rDNA (*internal trancriber spacer* do DNA ribosomal).

O trabalho está organizado nos seguintes itens: 1- Introdução Geral; 2- Objetivos; 3- Material e Métodos; 4- Resultados (dividido em seis Capítulos); 5- Síntese; 6- Referências Bibliográficas e 7- Anexos.

O item "Material e Métodos" contém detalhes das áreas de coletas, métodos de coleta e armazenamento de DNA em FTA-Card, métodos de identificação morfológicos dos macrofungos, sequenciamento da região ITS rDNA e estratégias para abordagem dos dados moleculares.

Os capítulos de resultados estão dispostos em formato de artigos científicos da seguinte forma:

CAPÍTULO 1: resultados obtidos pelo estudo ecológico, que aborda a diversidade de macrofungos Agaricales em floresta primária e secundária na área de manejo florestal. Artigo submetido à Revista Biotropica;

CAPÍTULO 2: descrição de seis espécies novas de *Tetrapyrgos* pela inferência de dados morfológicos e moleculares. Artigo submetido à Revista Mycologia, atualmente aceito para revisão;

CAPÍTULO 3: descrição de uma espécie nova de *Marasmius* que sugerimos ser próxima da espécie *M. horridulus* que é a única espécie dentro da subsecção *Horriduli*, descrita por Singer em 1986. Este trabalho utilizou dados morfológicos e moleculares e será submetido à Revista Phytotaxa;

CAPÍTULO 4: descrição de espécies de *Marasmius* que apresentam rizomorfos e breve discussão sobre esse caracter não distintivo para separar os grupos de espécies dentro da seção *Marasmius*. Artigo formatado para a Revista Acta Amazonica;

CAPÍTULO 5: descrição de espécies de *Marasmius* coletados em floresta de terra firme. Artigo formatado para a Revista Fungal Diversity;

CAPÍTULO 6: descrição de espécies da família Omphalotaceae, *Gymnopus*, *Rhodocollybia* e *Marasmiellus*. Artigo formatado para a Revista Acta Amazonica.

Os morfotipos referentes ao capítulo ecológico, não necessariamente correspondem à mesma designação nos artigos taxonômicos, uma vez que muitas espécies foram identificadas posteriormente e/ ou tiveram seu nome alterado para se adequar ao objetivo de cada trabalho. Todos os capítulos taxonômicos apresentam pranchas de imagens ou ilutrações das estruturas microscópicas.

As sequências genéticas foram obtidas durante o doutorado sanduiche no Laboratory of Molecular Systematics- LMS do Royal Ontario Museum – ROM em Toronto, Canadá. Estas sequências serão incluídas no GenBank após a publicação dos artigos e os espécimes coletados serão integralmente depositados nos Herbários INPA e no Royal Ontario Museum Fungarium (TRTC).

1. INTRODUÇÃO GERAL

Os fungos são os principais decompositores de matéria orgânica de origem vegetal nos ecossistemas florestais, atuando na ciclagem de nutrientes como fósforo e nitrogênio, limitantes para a produção primária em florestas tropicais (Lodge et al. 1996). Os fungos saprotróficos ou decompositores são diversos nas regiões tropicais e subtropicais, onde se acredita que os principais fatores que contribuem para sua diversidade estejam também relacionados à diversidade de hospedeiro e de habitats, além de fontes nutricionais em abundância (Lodge et al. 1995).

As estimativas atuais da diversidade de fungos estão entre 1,5 milhão (Hawksworth 2001) a 5,1 milhões (Blackwell 2011) de espécies, sendo que apenas cerca de 80 a 120 mil espécies estão descritas (Webster e Weber 2007; Blackwell 2011). Dentre as diversas localidades possíveis de se encontrar espécies de fungos ainda não exploradas pela ciência, estão os países onde o conhecimento sobre a diversidade fúngica e seus hospedeiros, habitats e nichos ainda são escassos ou pouco estudados (Hawksworth 2001; Hyde 2001). Este é o caso dos países por onde se estede a floresta Amazônica.

No Brasil foram catalogadas apenas 5.711 espécies de fungos, segundo dados da lista de espécies da Flora do Brasil – 2016 (Maia e Carvalho 2016), número muito distante do estimado para as áreas tropicais, que é em torno de 35 mil espécies somente para os fungos macroscópicos (Mueller et al. 2007). Além disso, essa estimativa geralmente é feita com base na diversidade proporcional de plantas, justamente pela carência de estudos taxonômicos e ecológicos de fungos em áreas sul e centro tropicais (Mueller et al. 2007).

A Amazônia é a região com maior extensão de floresta tropical do mundo. É um bioma com características únicas, formado por vegetação de grande porte e densa, incluindo também mosaicos de vegetação conhecidas como campina e campinarana (Prance 1975; Lisboa 1975) e áreas alagáveis como a várzea e o igapó (Junk e Piedade 2010). Estes ecossistemas abrigam uma enorme diversidade de fungos macroscópicos. No entanto, o conhecimento dessa diversidade é muito incipiente, apresentando um grande descompasso no número de projetos e especialistas e um número subestimado de espécies existentes no bioma. E ainda estudos ecológicos em áreas tropicais que avaliam o comportamento da comunidade fúngica em locais de sucessão secundária da vegetação são escassos (Chaverri e Vílchez 2006).

A floresta amazônica, por oferecer grande quantidade de recursos naturais, é um dos biomas que vem sofrendo grande pressão ambiental e o uso sustentável que leva em conta

fatores social, econômico e ecológico é extremamente importante na proteção da floresta (Gardner et al. 2013),

Assim, a Estação Experimental de Manejo Florestal do Instituto Nacional de Pesquisas da Amazônia -INPA, onde o projeto BIONTE (Higuchi et al. 1997) realiza estudos de manejo florestal visando a pratica de retiradas de árvores de interesse madeireiro com menor impacto se mostrou interessante para se desenvolver estudos sobre a diversidade e ecologia de macrofungos. É uma área de floresta de terra firme, na qual há áreas em processo de regeneração (floresta secundária) próximas às áreas de florestas primárias, nas quais vários estudos já foram e continuam sendo realizados relacionados à biomassa e estoque de carbono.

Diante deste contexto, este trabalho teve por objetivo caracterizar a comunidade dos macrofungos Agaricales na dinâmica das florestas amazônicas em área de manejo florestal (floresta primária e secundária), utilizando uma abordagem filogenética e ecológica de estrutura de comunidade. Esse tipo de estudo tem se mostrado interessante para melhorar o entendimento na composição de comunidades, funcionamento dos ecossistemas e suas respostas frente às mudanças ambientais. Este trabalho aborda então, essas mudanças derivadas de ações antrópicas, no caso, corte de árvores.

Além disso, cabe ressaltar que o trabalho prezou a importância da identificação taxonômica morfológica aliada à identificação molecular de macrofungos Agaricales na Amazônia, uma vez que ainda há uma grande lacuna de conhecimento micológico, frente à enorme diversidade que se é estimada, sem falar que é imprescindível esta etapa para a base de trabalhos ecológicos.

1.1 Agaricales

Os cogumelos pertencentes a essa ordem apresentam uma variedade de formas, desde fungos lamelados às formas reduzidas e clavarioides, não apresentando então nenhuma característica morfológica como sinapomorfia do grupo (Matheny et al. 2007). Assim, os modos de nutrição dos fungos têm se mostrado como caracteres filogeneticamente mais informativos para a caracterização de diversos clados (Moncalvo et al. 2002; James et al. 2006).

Agaricales é monofilético e apresenta aproximadamente 8500 espécies, assim formando o maior clado em Agaricomycetes (=Homobasidiomycetes *sensu* Hibbett e Thorn 2001; Binder et al. 2005). Desta forma, o clado contendo predominantemente gêneros e famílias da subordem Agaricineae (*sensu* Singer1986) representa o que atualmente se considera Agaricales *sensu stricto* (*sensu* Moncalvo et al. 2002; Matheny et al. 2007).

Uma das principais obras taxonômicas de referência para o estudo de Agaricales é "The Agaricales in modern taxonomy" (Singer 1986), na qual foram organizados 230 gêneros em 18 famílias. Nessa abordagem, três grupos fazem parte de Agaricales s.l.: Agaricales s. str., Boletales e Russulales. Esses três atualmente formam os clados "eugaricus", "boletes" e "russuloid" segundo análises filogenéticas com base em dados moleculares (Hibbett e Thorn 2001). A filogenia molecular tem revelado que Agaricales *sensu* Singer apresenta uma estreita equivalência com o clado euagaricus (Hibbett et al. 1997; Moncalvo et al. 2000; 2002).

Moncalvo et al. (2002) realizaram um estudo filogenético com base em sequências do marcador nuclear LSU para entender a relação entre famílias e gêneros dentro de Agaricales. Estes autores então sugeriram 117 clados, dentro os quais foram revelados o monofiletismo de algumas famílias tradicionalmente aceitas. Moncalvo et al. (2002) de fato não resolveram tudo.

Já Matheny et al. (2006) em um trabalho de reconstrução filogenética multilocus, utilizaram seis regiões gênicas (*rpb*1, *rpb*1-intron 2, *rpb*2 e os genes 18S, 25S e 5.8S do RNA ribosomal) e 49% dos gêneros de Agaricineae *sensu* Singer (1986) foram representados e sugeriram informalmente seis principais clados: "Agaricoid", "Tricholomatoid", "Marasmioid", "Pluteoid", "Hygrophoroid "e "Plicaturopsidoid".

Recentemente, Dentinger et al. (2015) com o objetivo de avaliar as relações evolutivas em Agaricales com dados mais robustos, obteve alto suporte para os nós mais profundos, utilizaram o genoma de 39 espécies de 26 famílias, com isso foi possível o reconhecimento de sete subordens: Agaricineae, Pluteineae, Tricholomatineae, Marasmineae, Schizophyllineae, Pleurotineae e Hygrophorineae.

1.2 Agaricales na Amazônia

Na Amazônia, Rolf Singer foi uns dos pesquisadores que mais realizou estudos com macrofungos durante o século passado. Descreveu inúmeras espécies novas de Agaricales para a região, sendo dois gêneros novos de Tricholomataceae s.l.: *Pegleromyces* Singer e *Callistodermatium* Singer (Singer 1981). Outras espécies descritas para região de Manaus pertencem aos gêneros *Hygrocybe* (Fr.) P. Kumm, *Collybia* (Fr.) Staude, *Marasmiellus* Murrill, *Hohenbuehelia* Schulzer e *Oudemansiella Speg*., dentre outros (Singer 1989). *Entoloma* Fr. ex P. Kumm. e *Marasmius* Fr. são os gêneros amazônicos de Agaricales com maior número de espécies (Lista de espécies flora do Brasil, 2016).

Os estudos filogenéticos de Agaricales ainda carecem de amostras de espécies tropicais. Podemos exemplificar a família Tricholomataceae s. str., a qual apresenta muitas

espécies descritas por Singer e não foram recoletadas e muito menos fazem parte de estudos de filogenia. *Callistodermatium violascens* Singer, *Lulesia densifolia* Singer, *Mycoalvimia theobromicola* Singer, *Neoclitocybe byssiseda* (Bres.) Singer, *Pegleromyces collybioides* Singer são exemplos desses táxons tropicais pouco conhecidos, alguns dos quais coletados somente no Brasil.

Em relação aos aspectos ecológicos de macrofungos Agaricales, somente alguns estudos foram realizados na região. Na Reserva Walter Egler em Manaus-AM, Brasil a diversidade de macrofungos foi avaliada por Souza e Aguiar (2004), que estudaram a diversidade de Agaricales e compararam a distribuição das espécies nas áreas de platô, vertente e baixio. Além disso, elas observaram características do substrato em que as espécies se encontravam. Neste trabalho foram ainda observadas a predominância de fungos na área de platô e a ocorrência predominante de fungos lignícolas.

Já no estudo de Braga-Neto et al. (2007) a diversidade de fungos marasmióides de serrapilheira na Reserva Ducke, Manaus-AM, Brasil foi analisada em comparação com a distribuição temporal e espacial nesse ambiente, sugerindo que a topografia e estrutura da vegetação têm influencia na distribuição e produção dos basidiomas em períodos de baixa pluviosidade.

2 OBJETIVOS

A partir do que foi exposto sobre os estudos referentes aos macrofungos na Amazônia, há muito para se conhecer sobre a diversidade fúngica e seus aspectos taxonômicos, ecológicos e filogenéticos. A proposta deste trabalho foi:

Avaliar quais são as espécies de macrofungos Agaricales que ocorrem na floresta de terra firme em área de manejo florestal e se a composição e riqueza da comunidade de macrofungos variam de acordo com a sazonalidade e a idade das florestas (florestas primárias e secundárias) em escala local.

2.1 Objetivos específicos

1. Realizar a identificação taxonômica morfológica dos espécimes de Agaricales previamente coletados na Estação Experimental de Manejo Florestal do INPA (ZF2) em área de floresta secundária e primária;
2. Realizar coletas em diversas áreas de floresta de terra firme na amazônia para incluir espécimes no trabalho taxonômico.

2. Atualizar a descrição das espécies de fungos marasmioides e gymnopoides, ou mesmo identificação de possíveis espécies novas para a ciência;
3. Realizar a caracterização molecular dos espécimes coletados por meio de sequenciamento da região ITS (ITS1, 5.8 S e ITS2).

3 MATERIAL E MÉTODOS

3.1 Características e histórico da área de coleta

A principal área de estudo foi a Estação Experimental de Manejo Florestal do INPA no km 23 da estrada vicinal ZF-2, cerca de 80 km ao Norte de Manaus (coordenadas geográficas: S 02°37' a 02°38' e W 60º09' a 60°11').

A vegetação da área corresponde à Floresta Densa de Terra Firme Amazônica, com uma composição florística bastante heterogênea (Jardim e Hosokawa 1986/87). Guillaumet (1987) distinguiu quatro estratos verticais: árvores acima de 15 m de altura, árvores de 12-15 m, árvores pequenas e arbustos de 7-12 m e arbustos até 7 m.

O solo dos platôs da região corresponde ao Latossolo Amarelo, álico, textura muito argilosa, muito ácida, com alto teor de alumínio e baixa capacidade de troca catiônica. O clima é equatorial quente e úmido, com chuvas abundantes e bem distribuídas ao longo do ano, sendo a temperatura média do mês mais frio sempre superior a 18 °C e possuindo características de isotermia, ou seja, com amplitude térmica média anual (média do mês mais quente menos a média do mês mais frio) inferior a 3 °C; há uma curta estação seca, onde o menor índice pluviométrico (agosto) apresenta precipitação inferior a 100 mm e com chuvas mensais que ultrapassam 290 mm na época chuvosa (Ferreira et al. 2006).

Desde a década de 1980, o Instituto Nacional de Pesquisas da Amazônia - INPA vem desenvolvendo experimentos com manejo florestal, visando a produção sustentável de madeira. Em 1993, o projeto "BIONTE" - Biomassa e Nutrientes na Floresta Tropical Úmida, realizou um experimento com corte seletivo da floresta, com o intuito de estudar os efeitos ecológicos da extração seletiva de madeira e definir estratégias de corte seletivo sustentável de árvores (Ferreira et al. 2006).

O presente estudo foi realizado dentro da área do projeto BIONTE (Higuchi et al. 1997), onde as parcelas controle (200 × 200 m^2) dos blocos I e II foram selecionadas para montar os transectos na floresta primária. Essas áreas foram selecionadas, pois há um estudo para identificação das espécies de plantas. As áreas de florestas secundárias adjacentes a essas áreas são resultado de corte total de madeira para estudo de biomassa e estoque de carbono,

com aproximadamente 27 anos de regeneração (Higuchi, com. pess.). Essas áreas são compostas principalmente por espécies de Melastomataceae (*Miconia tomentosa* (Rich.) D. Don ex DC. e *Bellucia dichotoma* Cong.), *Cecropia* sp. e Rubiaceae, que não ultrapassam diâmetro médio de 15,19 cm (Komura, obs. pers), diferentemente da área primária, no qual se observa uma quantidade muito mais expressiva de espécies de plantas (Gaui 2013) com isso contribuindo com a heterogeneidade e com uma camada mais espessa de serrapilheira.

Outras áreas de coletas foram:

(1) Campus do Instituto Nacional de Pesquisas da Amazônia- INPA (3° 05' 47" S, 59° 59' 14" W) em Manaus;

(2) Reserva Biologica Ducke- INPA (2° 58' 48" S, 60° 09' 08" W), na vizinhança de Manaus;

(3) Reserva de Desenvolvimento Sustentável do Tupé (3° 07' S, 60° 18' W), 25 km oeste de Manaus seguindo pelo Rio Negro;

(4) Comunidade São Sebastião (2° 48' 04" S, 60° 29' 58" W), localizado no rio Cuieras, braço do Rio Negro em Novo Airão;

(5) Reserva Biológica do Uatumã em Balbina, ao norte de Manaus (S1°47'21" S, 59°15'08" W);

(6) Cachoeira da Iracema, em Presidente Figueiredo (1° 59' S, 60° 03' W), 100 km ao norte de Manaus;

(7) Baixo rio Aracá (0° 07' S, 63° 19' W), aproximadamente a 400 km noroeste de Manaus;

(8) Parque Nacional do Viruá em Caracaraí, Roraima (1°28'05"N, 61°00'32" W);

(9) Floresta Nacional do Tapajós (2° 51' 37" S, 54° 57' 57" W) na base Terra Rica do ICMBio localizado no km 67 da rodovia BR-163 e comunidade Jamaraquá (2° 49' 45" S, 55° 01' 56" W), Belterra, Pará.

3.2 Procedimentos de coleta e identificação dos macrofungos Agaricales

Os macrofungos Agaricales que eram facilmente detectáveis sobre o solo e serrapilheira foram coletados. Registros fotográficos dos macrofungos Agaricales e anotações quanto ao substrato, subplot foram realizados no campo.

Os materiais coletados foram separados em morfotipos e parte identificados com base em bibliografias e chaves de identificação disponíveis para Agaricales tropicais e em parceria com os pesquisadores Dr. Jadson J. S. Oliveira, Dr. Felipe Wartchow (Universidade Federal da Paraíba), Dra. Maria Alice Neves (Universidade Federal de Santa Catarina) e Dr. Jean-

Marc Moncalvo (Department of Natural History, Royal Ontario Museum e Department of Ecology and Evolutionary Biology, University of Toronto, Canadá).

Os dados referentes aos nomes das espécies tiveram como base o Index Fungorum (http://www.indexfungorum.org) e o MycoBank (http://www.mycobank.org).

As principais bibliografias para os termos utilizados na descrição foram Fidalgo & Fidalgo 1967, Largent 1973, Largent et al. 1973 e Lodge et al. 2004. Para a descrição e identificação das espécies foram utlizadas Dennis 1951, Singer 1965, 1973, 1976, 1986, 1989, Tan et al. 2009 e Wannathes et al. 2009, além de outras referências que são citadas em cada capítulo.

3.3 Armazenamento das amostras em Whatman FTA® cards no campo

As amostras para o estudo molecular dos espécimes foram armazenadas em FTA card a partir do cogumelo ainda fresco, do qual retirou-se um fragmento (dependendo do cogumelo, pode ser da lamela ou parte do contexto (parte mais interna do cogumelo)) limpo e livre de qualquer contaminação visível (Ex. manchas, terra, folhas, etc) utilizando pinça ou lamina de barbear esterilizada com álcool ou aquecida em chama de lamparina. O fragmento foi depositado no cartão FTA e com a cobertura do cartão fechada, foi aplicada uma pressão rápida e uniforme utilizando um martelo (Dentinger; Margaritescu; Moncalvo 2010). Este método pode ser observado com mais detalhes no video do link abaixo: https://www.youtube.com/watch?v=Gir56iYspTE.

3.4 Extração de DNA em FTA-card

Esta etapa, e as subsequentes referentes à extração, amplificação e sequenciamento, foram todos realizados Laboratory of Molecular Systematics- LMS no Royal Ontario Museum- ROM, Toronto, Canadá. Todos os reagentes necessários foram disponibilizados neste laboratório sem custo adicional.

A extração do DNA das amostras previamente armazenadas em FTA-card foi realizada seguindo as etapas abaixo:

a-) discos em torno de 2 mm de diâmetro de material fungico contido nos cartões Whatman FTA® cards foram cortados com cortador e foram depositados em placas de extração de 96 poços (95 materiais e um controle) (mais detalhes Anexo I: FTA card extraction method)

b-) Após certificar que cada poço continha apenas um disco (ou seja apenas uma amostra), adicionou-se: 25 µl solução de extração (Sigma) e mantido a 95 °C por 10 min; após isso

adicionou-se 25 µl solução de diluição (Sigma) para parar a reação e foi mantida em freezer -20 °C.

A partir desse processo obteve-se amostras de DNA para PCR (DNA-mãe). Para a reação de PCR utilizou-se uma aliquota de 5 µl de DNA-mãe para 45 µl de água destilada (Sigma), obtendo-se uma diluição de 1:10.

3.5 Extração de DNA a partir do material desidratado

Este método foi utilizado para os especimens que não foram possível o armazenamento por meio do FTA-card, ou para materiais os quais não obtiveram-se DNA para sequenciamento e para os materias de herbário.

O protocolo a seguir **"**DNA Extraction protocol in plates using glass fiber AcroPrep-PALL plates" é utilizado no Laboratório do ROM por Simona Margaritescu (Anexo II).

Uma pequena aliquota de amostra de fungo desidratado (~1.5 mm) foi colocada em cada poço da placa de 96 poços (do mesmo tipo da usada na extração a partir do FTA card), cobriu-se cada linha com capas próprias para esse tipo de placa. Para uma placa, misturou-se 5 ml de tampão de lise para Vertebrados e 0,5 ml de proteinase K (20 $mg.ml^{-1}$) em um tubo esterilizado. Adicionou-se 50 µl desse Mix de Lise em cada um dos poços das placa de 96 poços. A placa foi incubada a 56 °C por no mínimo 6 horas ou *overnight* para permitir a digestão dos tecidos. Após esses processo, a placa foi centrifugada por 30 s para remover qualquer material condensado na cobertura. Adicionou-se 100 µl do Mix de Ligação (tabela A) em cada poço e homogeneizando com a própria pipeta, centrifugou-se por 20 s para sedimentar o lisado. As coberturas foram removidas e os materiais lisados (150 µl) transferidos para poços de uma placa to tipo PALL (placa com filtro de fibra de vidro – AcroPrep 96 filter plate PN5051 1,0 µm glass), selou-se a placa com cobertura adesiva e acoplou-se o bloco de vácuo nessa placa, e por meio desta aplicou-se pressão com bomba de vacuo para filtrar esse material. O DNA fica retido nas fibras.

Após esta etapa, iniciou-se o processo de lavagem abaixo:

1-) Adicionar 250 µl de Protein wash buffer (PWB) em cada poço contendo o filtro de fibra de vidro. Selar a placa e aplicar pressão para filtragem;

2-) Adicionar 300 µl de Wash buffer (WB) em cada poço contendo o filtro de fibra de vidro. Selar a placa e aplicar pressão para filtragem;

3-) Adicionar 300 µl de Wash buffer (WB) em cada poço contendo o filtro de fibra de vidro. Selar a placa e aplicar pressão para filtragem;

* Se observado que o WB não atravessa a membrana, abra a cobertura adesiva e sele novamente.

Após a lavagem, removeu-se a cobertura, acoplou-se a placa em um suporte, cobrindo com lenço de papel e foi incubado a 56 °C por 30 min.

A placa de 96 poços foi acoplada com suporte abaixo da placa de filtro de vidro, utlizando fita adesiva para certificar que isso ficaria adequadamente encaixada. Dispensou-se 50 µl de ddH2O (preaquecida a 56 °C) diretamente na membrana de filtro de vidro em cada poço, incubou-se a temperatura ambiente por alguns minutos, após isso a placa foi selada.

Todo o aparato foi centrifugado a 1500 g por 10 min para que o DNA fosse eluído na placa abaixo da placa contendo a membrana de vidro. Desencaixou-se a placa de cima, esta foi descartada. A placa contendo o extrato de DNA foi coberta com fitas de tampas de plástico e mantidas estocadas em freezer a -20 °C.

O protocolo original e preparo das soluções utilizadas estão no ANEXO II: DNA Extraction protocol in plates using glass fiber AcroPrep-PALL plates.

3.6 Amplificação em PCR

A reação de PCR foi iniciada aliquotando os seguintes reagentes: 4,9 µl de Água destilada (Sigma); 1,0 µl de 10 × tampão EH; 1,6 µl de 1.25 µM dNTPs; 0,2 µl de 10 µM ITS1; 0,2 µl de 10 µM ITS4; 0,1 µl de **Platinum® *Taq* DNA Polymerase**; 2,0 µl de DNA mãe (1:10), totalizando 10 µl de volume total por reação.

No termoclicador utilizou-se a seguinte programação de PCR: temperatura de desnaturação 94 °C por 4 s; anelamento 55 °C por 5 s e polimerização 72 °C por 1 mim. Esse ciclo foi repetido por 35 ×.

A verificação e seleção dos resultados dos produtos da PCR foram visualizados em Gel de agarose. Para tal, foi preparado um gel de agarose 1% (100 ml 1 × TA e 1 g agarose). Esta quantidade foi suficiente para uma placa utilizando dois pentes de 1,5 mm com 24 dentes e assim, dois géis foram preparados para correr todas as amostras.

Após fusão em micro-ondas do gel de agarose, adicionou-se 10 µl de SYBR Safe (DNA Gel Strain) e este foi despejado em placa de gel, com dois pentes de 24 poços e aguardou-se até sua total solidificação.

Nas amostras de produto de PCR adicionou-se 5 µl de 18% FICOLL.

O conjunto de gel foi colocado na cuba de eletroforese, cobrindo com o tampão. As amostras foram dispostas nos poços do gel. A voltagem de corrida foi ajustada para 100 mV. Ao final da corrida, as bandas de DNA foram visualizadas em luz azul.

As bandas que se mostraram bem definidas ou únicas foram cortadas com laminas e depositadas em tubos de 2 ml contendo filtro de purificação. Nesta etapa utilizou-se o método descrito com mais detalhes no ANEXO III. Os tubos foram centrifugados por 10 min a 14220 g ou 13.200 rpm. Os filtros foram descartados e o DNA filtrado a partir do gel foram mantidos em freezer -20 °C.

3.7 Reação de Sequenciamento

Para a reação de sequenciamento utilizou-se os seguintes reagentes, por reação: 0,5 µl de Big Dye; 1,0 µl de BD Buffer; 1,0 µl de Primer (10 µM); 2,0 µl de Betaine (5M); 1,5 µl de água destilada (Sigma); 4,0 µl de DNA filtrado da etapa anterior.

Os dois primers utilizados foram ITS1-F (*forward*) e ITS4 (*reverse*) (White et al. 1990, Gardes & Bruns 1993). Estes dois primers cobrem a região ITS1, 5.8 S e ITS2, como mostra o esquema da Figura 1.

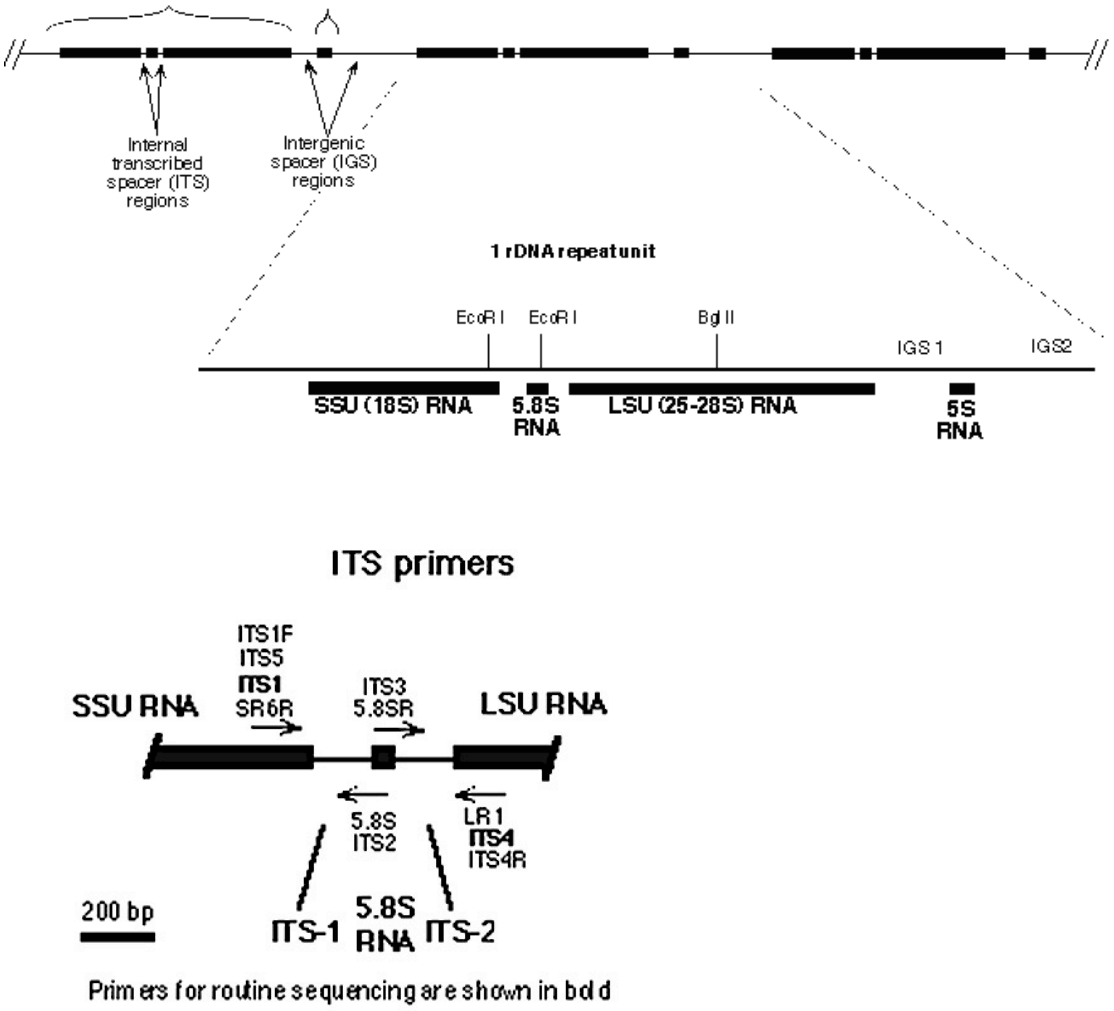

Fig1. Localização da região do ITS (ITS1–5.8S–ITS2) sequenciada e primers utilizados (ITS1 e ITS4). Fonte: http://sites.biology.duke.edu/fungi/mycolab/primers.htm

O programa de reação de sequenciamento BigDye_KC do termociclador foi utilizado, 1 ciclo de 96 °C por 1 min; 30 ciclos de 96 °C por 10 s, 50 °C por 5 s, 60 °C por 4 min; e 1 ciclo de 60 °C por 5 min. Após o processo, a placa foi mantida coberta com papel aluminio (evitar exposição à luz), e em freezer -20 °C, até o processo de limpeza dos produtos de sequenciamento.

A limpeza dos produtos de sequenciamento foi realizada pelo "Método de precipitação em álcool". Para esse processo o material foi tranferido para uma placa especial de leitura no sequenciador, placa optica MicroAmp® Optical 96-Well Reaction Plate (ABI-plate). Assim, misturou-se volumes iguais de 125 mM EDTA e 3 M Acetato de sódio e seguiu se as etapas abaixo:

a-) 2 µl da mistura acima de EDTA e NaOC por poço;

b-) Transferir os 10 µl dos produtos da reação de sequencimento para a placa ABI;

c-) Adicionar 25 µl de etanol 100% por poço;

d-) Incubar à temperatura ambiente (~25 °C), no escuro, por 15 min;

e-) Balancear a placa para centrifugar a 4 °C, 2500 g por 30 min;

f-) Retirar a placa, cobrir com papel toalha e inverter a placa e centrifugar a 100 g por 1 min, para se retirar a parte liquida;

g-) Remover a placa da centrifuga e aliquotar 35 µl de etanol 75% por poço;

h-) Centrifugar a 4 °C, 2500 g por 15 min;

i-) Repetir o passo f;

j-) incubar a placa, descoberta no termociclador a 95 °C por 1 min;

Continuando:

Ressuspender em 10 µl Hi-Di Formamida, desnaturar coberto com a *septa cover* (é uma cobertura de borracha cinza) no termociclador a 95 °C por 2 min; e manter em suporte gelado ou em freezer ao abrigo da luz até ser analisado no sequenciador.

Os materiais foram sequenciados no ABI-3730- DNA Analyzer (Applied Bio-systems– Hitachi).

3.8. Edição, alinhamento das sequências e análises filogenéticas

As sequencias obtidas foram manualmente editadas utilizando o software Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, MI). Os contigs obtidos foram comparados com banco de dados do GenBank (http://blast.ncbi.nlm.nih.gov/) por meio do BLAST (Altschul et al. 1990). Para filtrar os dados das sequências obtidas pelo GenBank, utilizou-se o script gb2fasta.pl

(https://sites.google.com/site/santiagosnchezrmirez/home/software/perl).

Os alinhamentos foram realizados utilizando MUSCLE (Edgar 2004) e ajustes manuais foram realizados por meio do programa MEGA version 6 (Tamura et al. 2013).

Os modelos evolutivos foram inferidos por meio do programa jModeltest 2.1.7 (Darriba et al. 2012). A análise de Máxima Verossimilhança foi realizada utilizando os parâmetros obtidos no JModeltestos e os valores de *Bootstrap support* (BS) para as ramificações foram estimadas a partir de 1.000 replicações.

As analyses Bayesianas foram realizadas por meio do portal CIPRES (https://www.phylo.org/portal2/login!input.action) e os parâmetros utilizados são detalhados no capítulo em que foi utilizado esta análise.

4- RESULTADOS

Durante o projeto foram coletados 1070 espécimes (Tabela 1), sendo 669 espécimes nos transectos dispostos na área do Projeto BIONTE, localizado na Estação Experimental de Manejo Florestal (ZF-2). Além disso, foram coletados espécimes localizados na mesma área do projeto, mas que se encontravam fora dos transectos (231 espécimes) e estes não foram contabilizadados para o trabalho ecológico.

Outras oportunidades de coletas surgiram durante o projeto e assim as áreas descritas previamente no item Materiais e Métodos foram incorporadas no projeto para a etapa taxonômica.

A partir das coletas dos macrofungos Agaricales para o trabalho ecológico e nas demais áreas verificou- se uma grande diversidade de fungos marasmioides e gymnopoides, para os quais um estudo taxonômico detalhado foi realizado.

Tabela 1 – Número de espécimes coletados por localidade durante o projeto

Locais de coleta	Número de espécimes
Transectos	669
ZF2 (fora dos transectos)	231
Outras localidades de Terra Firme	170
Total	1070

Este trabalho presou os aspectos ecológicos aliados às análises taxonômicas, isso levou de início, a identificação e descrição de cinco espécies novas (Capítulos 2 e 3) para a área de manejo florestal. O que levantou algumas questões: As áreas de manejo florestal

permitem que espécies de macrofungos sejam conservadas? Haveria alguma relação entre a preservação da floresta e descoberta de espécies novas de fungos? Os fungos estariam ameaçado com a crescente onda de desmatamento?

Talvez este trabalho não seja capaz de responder a todas essas questões, mas é capaz de nortear alguns pontos. Uma análise preliminar mostrou que três das quatro espécies novas de *Tetrapyrgos* ocorreram tanto na floresta primária, quanto na secundária, enquanto que uma espécie nova de *Tetrapyrgos* e outra de *Marasmius* somente foram registrados na floresta primária. Em relação às espécies de *Marasmius* que apresentam uma estrutura de rizomorfo ereta, estas espécies foram exclusivas de floresta primária, não só na ZF-2, mas nas demais áreas estudadas (capítulo 4).

Isso pode indicar que algumas espécies poderiam ser mais resistentes à perturbação do ambiente, que é o caso de algumas espécies de *Tetrapyrgos* e *Marasmius* (Lodge & Cantrell 1995) e outras poderiam estar mais ameaçadas com a modificação drástica do meio, no caso, espécies de *Marasmius* com rizomorfos. O trabalho com essas espécies continua em andamento, mas é possível sugerir que pelos menos três espécies são novas.

Parece óbvio que a conservação das florestas permite a conservação dos macrofungos, no entanto, essa relação não é tão simples, uma vez que os fungos, embora tenham a exigência por umidade em comum para o crescimento, eles apresentam diferentes níveis de resiliência e colonização de um nicho, o que faz com que espécies "menos resistentes" corram maior risco de extinção antes mesmo de serem formalmente descritas pela ciência. Assim um dos pontos que pretendemos destacar foi essa relação entre a conservação das florestas e conservação da diversidade de macrofungos.

Outro viés deste projeto foi estudar a taxonômia dos macrofungos por meio de caracteres morfológicos (macroscópicos e microscópicos) e molecular com o sequenciamento do ITS, que é a sequência preconizada como *barcoding* para fungos (Schoch et al. 2012).

Para isso, o DNA dos macrofungos foram armazenados por meio do FTA-card. A Tabela 2 mostra o número de sequências obtidas por este método e pelo método tradicional a partir da extração de DNA de espécimes desidratados de macrofungos Agaricales.

Os materiais armazenado em FTA-card que não tiveram a amplificação do DNA foram provenientes de espécies que no momento do armazenamento estavam muito desidratadas ou que apresentavam uma estrutura mais "fibrosa" e rígida como foi o caso de *Caripia montagnei* (5 amostras), *Marasmius*, *Marasmiellus* e *Gymnopus*. Isso pode ser devido ao armazenamento incorreto desses materiais impossibilitando o sequenciamento posterior.

Assim, as espécies que apresentavam uma morfologia pouco distintiva e que eram de interesse para os trabalhos taxonômicos e filogenéticos, foram submetidas ao método tradicional de extração de DNA para obtenção de suas sequência de ITS.

Tabela 2 – Número de espécimes sequenciados a partir de material em FTA-card e a partir dos espécimes desidratados coletados em várias áreas

	Método	
Status das sequências	FTA-card	Extração a partir dos espécimes secos
Quantidades de amostras	514	31
ITS obtidas	401	20
Ambíguas, precisam ser clonadas	85	7
Não amplificadas	28	4

Em geral, o método de armazenamento de DNA em FTA-card foi eficiente para obtenção de sequências e economia de tempo em laboratório quando comparado com o método tradicional.

A seguir, os resultados e discussões deste projeto são apresentados em capítulos formatados de acordo com as normas de cada revista a que os resultados foram submetidos ou que se pretende submeter.

Capítulo 1

Komura, D.L; Moncalvo, J. M.; Bento, L. S.; Neves, M. A.; Zartman, C. E. How do seasonality, substrate and management history influence macrofungal fruiting assemblages in a central Amazonian Forest?

Manuscrito submetido para *Biotropica*.

LRH: Komura, Moncalvo, Bento, Neves, and Zartman

RRH: Macrofungal communities in managed Amazonian forests

How do seasonality, substrate and management history influence macrofungal fruiting assemblages in a central Amazonian Forest?

Dirce Leimi Komura[1]

Jean-Marc Moncalvo[2]

Larissa S. Bento[1]

Maria A. Neves[3]

&

Charles E. Zartman[1*]

[1]Instituto Nacional de Pesquisas da Amazônia, Dept. of Botany, Manaus, Amazonas, Brazil;

[2]Dept. of Natural History, Royal Ontario Museum, and Dept. of Ecology and Evolutionary Biology, University of Toronto, Canada;

[3]Dept. of Botany, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil.

* Corresponding author e-mail address: chaszartman@gmail.com

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ABSTRACT

High tree diversity, a trademark of Amazonian rainforests, is correlated with macrofungal diversity due to the resource heterogeneity that lignaceous plants provide for decomposer communities. Since fungi are responsible for much of this decomposition, understanding the factors which structure macrofungal communities should provide insights into predicting changes in global carbon stocks in light of deforestation and climate change. We investigate variation in community structure of fruiting (basidiome) macrofungi (Agaricales) in light of seasonality, forest history and substrate type. The study was conducted at the Biomass and Nutrient of Tropical Rain Forest (BIONTE's) project located in Central Amazonia where basidiome surveys of four substrate classes were conducted in permanent 250m^2 transects for two-years (2012–13) in managed and unmanaged forests. From the 669 fruiting bodies collected, 287 taxa were recorded of which 44% were restricted to primary and 36% to managed forest plots. Despite the fact that species-accumulation curves did not reach the asymptote, rarefaction analyses and Fishers alpha indicate no overall differences in richness among forest types. Differences were rather related to seasonality as secondary forests harbored disproportionately lower basidiome richness and abundance relative to primary forests in dry months. Basidiome richness of leaf litter guilds was higher in managed forest plots, whereas, in contrast, soil guild richness was greater in primary forest. These results suggest that the effects of forest management on fruiting of Amazonian macrofungi may be guild-dependent, and contrasting effects are likely due to differences in the quality and density of necromass correlated with to disturbance gradients in Amazonian forests.

Keywords: Agaricales; Amazon; Brazil; forest management; fungi; rainforest; rarefaction

ABSTRACT IN PORTUGUESE

A grande diversidade de plantas, característica típica da Amazônia, é um dos fatores que influenciam a composição de fungos por proporcionar fontes heterogêneas de material para a comunidade de organismos decompositores. Sendo os fungos um dos responsáveis pela ciclagem de matéria orgânica, o entendimento dos fatores que influenciam seu surgimento e distribuição, ainda que local, pode nos fornecer informações sobre as mudanças do fluxo de carbono e nitrogênio em âmbito global. O objetivo deste trabalho foi investigar a variação da estrutura da comunidade de macrofungos Agaricales em relação à sazonalidade, idade das florestas e tipo de substrato. Este estudo foi realizado na área de manejo florestal do Projeto BIONTE (Biomass and Nutrient of Tropical Rain Forest) na Amazônia Central, quando florestas primárias e secundárias foram utilizadas para monitorar, coletar e identificar macrofungos Agaricales durante o período de 2012-13. As coletas foram realizadas em oito transectos permanentes de 250m^2 cada, e quatro classes de substratos foram levados em conta. Como resultado, dos 669 macrofungos coletados, 287 espécies/morfoespécies foram identificadas, sendo 44% restritas à floresta primária, 33% à floresta secundária e 20% encontradas em ambas as florestas. Da mesma forma, a composição de macrofungos foi diferente entre as duas florestas, diferindo também de acordo com a estação e o ano de coleta. A detecção de macrofungos foi muito baixa na estação seca, principalmente na floresta secundária. Observou-se que, em relação aos substratos (troncos, galhos, folhas e solo), a floresta primária apresentou maior índice de riqueza e diversidade para solo. Por outro lado, no substrato folha, o índice de diversidade e de riqueza foi maior para floresta secundária.

INTRODUCTION

FUNGI ARE IMPORTANT COMPONENTS OF TROPICAL FORESTS DUE TO THEIR FUNDAMENTAL role in nutrient cycling dynamics and primary production (Lodge *et al*. 1996). However, their metabolic activity and fruiting regimes are affected by changes in temperature and humidity (Lodge & Cantrell 1995). Warming trends in temperate regions have repeatedly been correlated with shifting fruiting times in associated macrofungi communities (Gange *et al*. 2007, Kauserud *et al*. 2010). However, little is known about climate effects on tropical and, more specifically, Amazonian fungal fruiting phenology. Global climate models predict that annual precipitation will decline across large sections of Amazonia during the next decade (Cai *et al*. 2015, Li *et al*. 2011, Lewis *et al*. 2010, Harris *et al*. 2008). Furthermore, soil fungi and those of their associated tree communities are demonstrably coupled across western Amazonia (Peay *et al*. 2013). Assuming the climatic models are accurate, the capacity of fungi to recycle vegetal necromass may be compromised as the synergistic effects of drying trends and changes in plant community structure are expected to confer unpredictable consequences on the complex interactions among these aspects of Amazonian biodiversity.

Forest history is an additional complicating factor potentially altering, in unpredictable ways, the dynamics of plant-fungal associations in tropical biomes. Relatively well documented in Amazonia, for example, are the impacts of forest fragmentation on tree community structure and the subsequent cascade of effects on other groups (Laurence *et al*. 2011). The accompanying changes in above ground biomass, soil nutrient retention and tree regeneration capacity following deforestation are also well studied (Nascimento & Laurance 2004, Feldpausch *et al*. 2004, Bentos *et al*. 2013). Indeed, experimental studies of leaf litter characteristics conclude that comparative changes in decomposition rates among primary and secondary forests are principally due to differences in tree composition, and the correlated chemical and physical properties of their leaves which form the organic layer (Barlow *et al*. 2007, Vasconcelos & Laurence 2005, Luizão

1989). However, no studies have yet to provide complementary evidence on changes in Amazonian fungal communities in this context, and as a consequence, a fundamental piece of this puzzle is missing.

Extra-Amazonian studies of forest management practice impacts on macrofungal communities have been limited to specific groups (Chaverri & Vílchez 2006), focal host species (Tsui *et al*. 1998) or comparisons among heavily modified landscapes (e.g., monocultural tree plantations) (Paz *et al*. 2015). Nonetheless, the general consensus is that fungal diversity is inversely related to time since disturbance and positively related to that of the diversity of their associated plant communities. For example, in an eastern Costa Rica rainforest (Chaverri & Vílchez 2006) showed that hypocralean microfungi diversity was inversely proportional to stand age. In subtropical Brazil, exotic tree plantations of *Pinus* L. and *Eucalyptus* L'Hér. forest showed less similar macrofungal composition to native *Araucaria angustifolia* (Bertol.) Kuntze, forests than did monoculture stands of the same native species suggesting that the conversion to exotic tree plantations reduces the number of macrofungal taxa due to changes in substrate type and quality (Paz *et al*. 2015).

Surveying basidiomes is effective in identifying factors which may contribute to phenological changes of macrofungal communities in relation to environmental, historical or climatological factors (Gange *et al*. 2007, O'Dell *et al*. 2004, Lodge *et al*. 2004, Lodge & Cantrell 1995). However, basidiome surveys only provide a profile of the fruiting component of macrofungi assemblages. Such a "snap shot" image does not necessarily result in a comprehensive understanding of all species representative of the mycelial community in a given area. For example, in a north temperate forest stand in Canada, basidiome sampling resulted in a different species assemblage than those recovered from genetic screening of soil samples suggesting that soil fungi communities harbor a significant, cryptic component of temperate

forest fungal diversity (Porter *et al*. 2008). Nonetheless, active mycelial growth is a precursor to basidiome production suggesting that basidiome surveys, at the least, point to the subset of a species assemblage well established enough to allocate resources into sexual reproduction.

Another impediment to fungal community studies in tropical regions is the difficulty related to field species identification. Phenotypic convergence, plasticity, and a surplus of undescribed taxa from understudied regions all contribute to this challenge (Piepenbring *et al*. 2012, Braga-Neto *et al*. 2008). Most tropical fungi are ephemeral basidiomes producers, and this, combined with their exceptionally high diversity, precludes the comprehensive registry of taxa in a given locality without investment in intensive, long-term monitoring programs combined with genetic screening of the mycelial communities (Aime & Brearley 2012).

In this study, we conducted a two-year above ground basidiome survey in primary and selectively managed forest tracts in an upland rainforest of Central Amazonia in order to address the following hypotheses. Since nutrient fluxes in tropical forests are synchronized with wet and dry cycles (Lodge 1994), we hypothesize a strong association between plant and fungal communities. Likewise, anthropogenic factors may disrupt this association by, for example, altering soil quality through the compaction process (Hartmann *et al*. 2014) during timber extraction, or alteration of soil microclimate due to changes in canopy structure as the result of reduced tree densities (Lodge & Cantrell 1995).

Since tree species composition differs among the managed and primary forest plots at the study site (Gaui 2013), we hypothesize that the assemblage of macrofungal basidiomes varies according to forest management practice. Furthermore, secondary and edge-related forests of Central Amazonia are characterized by greater necromass production (Nascimento & Laurence 2004) resulting in greater density of rotting logs, related woody debris and forest floor leaf litter (Mesquita *et al*. 1997): keystone substrates for macrofungi in lowland rainforest. In light of these

demonstrable physical changes associated with the intensity of anthropogenic induced forest disturbance, we predict that macrofungal fruiting guilds, as defined herein by four substrate classes in lowland Amazonian forests (rotting trunks, branches, leaf litter and soil), may respond in independently and, perhaps contrasting manners relative to forest history. In summary, the aim of this study was to investigate at a local scale (10 km^2), whether seasonality, forest history, or the combination therein, promote contrasting effects on the diversity of four major fungal guilds in lowland tropical rainforests. Furthermore, by conducting repeated samples within the permanent study plots over the course of a two-year period, we set out to quantify, at a fine-scale (250 m^2), short-term temporal variation in Amazonian basidiome community structure.

METHODS

STUDY SITE.—The study area is located at the Experimental Station of the Forest Management (ZF-2) operated by Brazil's Instituto Nacional de Pesquisas da Amazônia (INPA) around 80 km north of Manaus, AM, Brazil (02°37' and 02°38' S; 60°09' and 60°11' W) (Fig. S1). The vegetation is upland Amazonian rainforest with high floristic heterogeneity. The soils are yellow latosol, with high clay content, acid with high quantities of aluminum and low capacity of cation exchange (Chauvel 1982). The climate is warm with high precipitation during the year, the driest month typically occurring in August (ca. 100 mm) and the rainiest months from February to June (> 290 mm) (Ferreira *et al*. 2006).

Since the 1980's INPA has developed forest management experiments with the goal of researching methods of sustainable wood production. In 1987, the project "BIONTE"- The Biomass and Nutrients of Tropical Rain Forest, performed experiments with selective wood extraction, to understand the ecological effects of this process (Ferreira *et al*. 2006). The present study used the experimental design of BIONTE's Project located at Experimental Station of the

Forest Management, which are composed by three blocks, each one with six plots with 200 × 200 m (Higuchi *et al*. 1997).

The collection sites present the same type of soil characteristics and altitude (Luizão *et al*. 2001), the difference between primary and secondary forest was the differences in age and tree composition. The secondary forest underwent a clear cut 27 yrs ago as part of the BIONTE experiment, and are primarily composed of Melastomataceae (*Miconia tomentosa* (Rich.) D. Don ex DC., *Bellucia dichotoma* Cogn.), Urticaceae (*Cecropia* sp., *Pourouma* sp.) and Rubiaceae tree, which do not go over 15.2 cm of mean diameter. The canopy is opened, allowing the light to reach the ground, which results in the desiccation of the substrate especially during the dry season. On the other hand, the primary forest canopy tree composition consists of a wider range of families, including Lecythidaceae, Leguminosae, Moraceae, Myrtaceae, Sapotaceae, Lauraceae, Burseraceae (Gaui 2013), thus contributing to high substrate heterogeneity. The understory, unlike secondary forests, is dominated by *Astrocaryum sciophilum* (Miq.) Pulle.

SAMPLING DESIGN.—Sampling plots were established as 5 × 50 m transects, divided in 10 subplots (5 × 5 m): four transects in primary forest (block I and II from the control plot of the Bionte's project); and four in nearby secondary forest (Fig. S1) totaling 40 subplots and eight transects. The fungi belonging to Agaricales were collected between May 2012 and September 2013 over six months during both years. Specifically, three consecutive months were collected during the height of the rainy (April-June), and dry (August-October) season resulting in a total of 12 inventories during the course of the study. To avoid temporal bias on basidiome detection, we visited in the same day one primary and one managed forest transect. Multiple basidiomes of the same species within each subplot or the same piece of substrate were counted as one occurrence. For each collection substrates were recorded and divided into the following classes:

trunk (> 10 cm diameter) buried and rooting, branches (up to 10 cm diameter), leaves (including petioles) and soil (found directly on humus). We did not find mushrooms growing on living trees.

TAXONOMIC IDENTIFICATION.— Basidiomes were described macroscopically in the field following Largent (1973) and Lodge *et al*. (2004), photographed, and subsequently dried using a food dehydrator and/or silicagel. Microscopic observations followed Largent *et al*. (1973) and specific literature according to the group. Specimens were separated into morphospecies and, when possible, identified to species. The literature used to identify specimens was mostly based on Singer (1986, 1976, 1964) as well as personal communication with specialists.

DATA ANALYSIS.—In order to quantify taxa diversity we used Fisher's alpha index (Magurran 1988) partitioned by substrate type and season, using the R Version 3.1.3 (R Development Core Team 2008) and R packages 'vegan' (Oksanen *et al*. 2013). Taxa accumulation curves were generated from the mean richness of 1000 randomized repeats of the sample ordered using the program EstimateS 5 (Colwell 1997). Rarefaction curves were based on a matrix of abundance data as the module estimates how many taxa you would expect to find at smaller sample sizes by conducting randomized subsets of the data, thus allowing statistically confident comparisons of taxa richness among samples of different sizes effectively decoupling density bias from richness. Community composition was summarized by principal coordinates analysis (PCoA) based on incidence data, presence or absence of taxa as well as number of occurrences data in each transect and according to forest treatment, substrate and year of collection. Bray-Curtis dissimilarity index was used as the distance measure.

RESULTS

BASIDIOME RICHNESS AND DIVERSITY IN RELATION TO SUBSTRATE, SEASON AND FOREST TYPE.—

During the two year survey, we recorded 669 basidiomes classified into 287 taxa (Table S1) of which 128 (44%) were exclusive to primary forests, 104 (36%) secondary forests, and 55 (20%) both forest types. Basidiome diversity (Table 1) and richness (Fig.1) varied among managed and primary forests depending on the substrate type. Whereas leaf-litter fungi had higher basidiome richness in managed forests, soil communities were greater in primary forests (Figs.1c and d). No significant difference among the two forest types for basidiome communities of rotting trunks and branches was detected (Figs.1a and b). Fisher's alpha corroborated with the richness values, as illustrated by the rarefaction curves, in all cases except for large necromass (leaves) where managed forests harbored greater basidiome diversity (Table 1). However, no overall differences in diversity were observed among managed and primary plots (Table 1).

Dry seasons resulted in sharp, concomitant declines in basidiome abundance and richness with the highest richness recorded in the rainy season and the lowest during the dry period (Fig.1e and f). These results were also supported by Fisher's alpha values (Table 1). The decline in richness and abundance in the dry season was disproportionately exacerbated in managed forests suggesting a synergistic effect of seasonality and forest history (Fig.1e and f). Whereas primary forest plots sampled during the dry season harbored 16% of the richness observed in the rainy season (175 compared with 29 taxa), secondary forests were comparatively less than half: 11% (156 compared with 18 taxa). This asymmetric decline in richness was accompanied by abundance as 15% of the observed rainy season basidiome numbers for primary forest compared to only 5% of those for managed forests.

A vast majority of the species restricted to one of the two forest types were only recorded during the wet season. Only eight and three of these species, primary and secondary forests respectively, were observed solely in the dry season. The same pattern was observed for shared taxa as only one of these 32 were restricted to dry season samples. No substantial deviations in

the ratio of primary forest restricted taxa relative to season and substrate were observed (Table S1), suggesting eliminated the possibility of asymmetric responses to fruiting phenology relative to seasonal in primary forests. There were too few basidiomes occurrences in dry season at secondary forests, so any interpretation was not possible.

SPECIES ACCUMULATION CURVES—A little more than half of the species (170; 59%) were singletons (e.g., a single basidiome recorded one time during the course of the survey), of which 79 (46.7%) were observed during first-year censuses, and 90 taxa (53.3%) in the second year. Rarefaction curves illustrate that a comprehensive sampling of fruiting Agaricales was not attained even when these data are partitioned by forest type, substrate and seasonality (Figs.1a-f). Furthermore, less than one-fifth of the 96 taxa observed in 2012 and 136 in 2013 were repeat observations among years. These included mostly common taxa, such as *Caripia montagnei* (Berk.) Kuntze, *Gloiocephala epiphylla* Mass., *Marasmius phaeus* Berk. & M.A. Curtis, *Mycena chloroxantha* Singer, *Mycena spinosissima* (Singer) Desjardin and *Tetrapyrgos longicystidiata*, A.H. Honan, Desjardin & T.J. Baroni which are shared among all forest plots. Most taxa belong to the genera *Marasmius* Fr. (79 spp.), *Mycena* (Pers.) Roussel (38 spp.) and *Gymnopus* (Pers.) Roussel (30 spp.), of which all inhabited either leaf litter or decaying branches. The most common morphospecies were *Mycena* "yellow1," *M.* "red1", and *M. spinosissima* (Singer) Desjardin, all of which were observed in all transects.

BASIDIOME COMPOSITION IN RELATION TO SUBSTRATE, FOREST TYPE AND YEAR.—Despite the fact that 81% (234) of all taxa were recorded as unique to either primary or managed forest plots (Table S1), substrate type overshadowed forest plot history in driving compositional variation (Fig. 2). Explained variance for PCoA axes 1 and 2 were similar for both abundance (24% and 23%,

respectively) and presence/absence data (23% and 24%) (Fig. S2), suggesting that differences in community composition are not attributable to changing abundance values of shared taxa.

The composition of soil and leaf-litter are more strongly conserved among both years and forest plot type respectively as illustrated by the fact that these two guilds show tighter clustering in ordination analyses than those for larger necromass such as downed branches and rotting trunks (Fig.2). Despite high annual species turnover, no accompanying shifts in basidiome community structure were observed between years (Fig.2). Temporal variation in fungal composition based on similar PCoA ordinations for fungal abundance resulted in explained variances of 27.7 and 18.9 % for axes 1 and 2 (Fig.2). In summary, soil macrofungi composition was more conserved across years when compared to the other two substrate types, leaf composition slightly less so and finally trunk and branch communities showed the greatest temporal variation in community structure among years.

DISCUSSION

Fungi are vital contributors to nutrient cycling and decomposition of organic material in forests worldwide (Smith & Red 1997) and they are an important, component of tropical biodiversity that are frequently cryptic (Arnold & Lutzoni 2007; Aime & Brearley 2012). However, little is known as to the sensitivity of tropical macrofungi to anthropogenic disturbances such as deforestation and climate change. By surveying experimentally managed and primary forests we made a first pass at understanding how fruiting phenology of four Amazonian understory fungal guilds vary in relation to rainfall and recent (< 30 yr.) anthropogenic disturbance.

During the course of a two-year intensive survey species–accumulation curves did not saturate for any of the focal fungal guilds in either forest condition. However, we provide suggestive evidence of a synergistic effect of seasonality and forest history on basidiome

community structure as dry season fruiting communities of managed forest plots harbored overall significantly lower abundance and richness than those of the primary forests. Furthermore, we observed guild-dependent contrasting effects of forest management practices on basidiome richness. Whereas managed forests harbored greater basidiome richness for leaf-inhabiting fungi they show a concomitant reduction in the soil community. This result suggests that changes in Central Amazonian basidiome community structure are possibly linked to the substrate quality and density which is likewise dependent on decaying processes influenced by disturbance regimes (Mesquita *et. al*. 1997; Feldpausch *et al*. 2004; Vasconcelos & Laurence 2005). Herein, we report guild-specific differences in basidiome richness among forest types, however, whether this is reflected in mycelial communities, as has been demonstrated in managed and unmanaged forests of Switzerland (Hartmann *et al*. 2014), remains unknown**.**

Only one-fifth of the taxa recorded in this study were shared among secondary and primary forest plots. However, whether high species turnover among forest types and survey year reflects changes in substrate quality brought about by forest management practices, or rather is artifactual due to incomplete sampling cannot be fully disentangled within the temporal and spatial framework of this study. Nonetheless, composition was well conserved within substrate types among forest types suggesting that niche shifts due to altered environmental conditions, as has been documented in other ephemeral cryptogamic communities in Central Amazonia (Zartman 2002), are not responsible for compositional changes among managed and primary forest plots.

SAMPLING INTENSITY AND TAXA SATURATION IN AMAZONIAN BASIDIOME COMMUNITIES.–The fact that species-accumulation curves are far from reaching asymptotes was not unexpected. Similar results from long term surveys in temperate regions on equivalent sized plots in Switzerland (21-year) and Austria (7-year) also showed that saturation was not attained (Straatsma *et al*. 2001;

Straatsma & Krisai-Greilhuber 2003). In tropical regions, such as Panama and the Colombian Amazon, shorter-term macrofungal basidiome surveys (2-year) conducted in both primary and secondary forests likewise showed that accumulation curves did not asymptote as well (Piepenbring *et al*. 2012, López-Quintero *et al*. 2012).

A majority of the recorded taxa in this study were singletons. This fact, in combination with the high fungal diversity and inter-annual taxa turnover explains why species-accumulation curves did not saturate. Likewise, in a nearby Central Amazonian forest Braga-Neto *et al.* (2008) observed that approximately 37% of the marasmioid fungi were repeats among surveys; however this study was only conducted over the course of one year so turnover may be more related to the seasonal differences in fungal fruiting phenologies. In addition, slightly less than 40% of the (morpho) species of *Marasmius* (a large genus of leaf litter fungi of lowland tropical forests) at Adolpho Ducke Ecological Reserve ca. 30 km distant from our study site were shared taxa (Braga-Neto *et al.* 2008; DLK, *pers.obs.*). Assuming that this high turnover over such a relatively short distance is repeated in other Agaricales taxa, the challenges of characterizing the distributions of Amazonian macrofungi are great. Although various authors have suggested that repeat surveys and diversifying sampling strategies may optimize species detection for cryptic and ephemeral organisms in general (Bills &Polishook 1994; Coddington *et al.* 1996; Sørensen *et al.* 2002; Yamashita *et al*. 2015) little remains known about macrofungal distribution patterns in tropical regions, and even less so in Amazonia.

GUILD DEPENDENT EFFECTS OF FOREST HISTORY ON FRUITING COMMUNITIES–Multivariate analyses from both presence-absence and abundance data suggest that substrate specific differences in macrofungi fruiting community structure overshadow those of management practice. Curiously, however a majority (80%) of the recognized taxa were mutually exclusive to either secondary or primary forest plots. This would suggest that overall composition of macrofungal communities varies a lot among primary and secondary plots sampled in this study. A similar result was found

for leaf litter fungi in which no correlation was found between vascular plant and macrofungal richness (McGuire *et al*. 2012). The importance of forest age on fungal composition is suggested to be linked to habitat differences associated with ontogeny of tree species and its subsequent effects on local micro-environments (Lodge & Cantrell 1995).

For example, Cowley (1970) showed that microfungal communities in decomposing leaf litter at Puerto Rico were sensitive to stress induced by canopy openings and irradiation that they no longer resembled communities on the same litter species in undisturbed forest. Thus, disturbances that disrupt the canopy contribute to spatial as well a temporal of decomposer fungi, particularly for basidiomycetes with superficial mycelia especially sensitive to drying (Hedger 1985). Likewise, changes in the physic-chemical properties of soils due to the compaction process of timber extraction have also been shown to be positively associated with some soil mycelial communities such as saprobes and parasites, whereas negatively associated to ectomychorrizal guild (Hartmann *et al*. 2014). However, our study showed a similar composition among soil fungi, this result can be under biasis once the number of collection was too low (91 basidiomes) and just six species shared among forest.

Soil and leaf litter fungal guilds varied less compositionally than that of trunks. In temperate regions, spatial aggregation has mostly been shown to be partitioned within, rather than among logs (Kubartová *et al.* 2012), suggesting that the composition of chance early colonizers in concert with the subsequent species interactions could results in a metacommunity dynamics such as quorum effects (Jenkins 2006) promoting complex geographic structure in species patch occupancy patterns (Gourbière & Gourbière 2002, Gilbert & Sousa 2002.). Likewise, macrofungal assemblages of large necromass, such as downed trunk and logs, undergo succession, which accompanies the stages of wood decay, that is, the quality of the substrate so does the composition of the decomposers (Allen *et al.* 2000). Proximity of primary forest may

also influence of fungal diversity in secondary forests as was observed in Costa Rica with Hypocreales fungi where no significant differences was found between 25-27 yr old forest and old-growth forest (Chaverri & Vílchez 2006), although we did not observed this pattern. For Naeem and Wright (2003), the complexity of rain forest regeneration, including the rapid accumulation of plant taxa in secondary succession in tropical forests, it seems likely that stochastic factors also have an influence in determining fungal taxa composition. However, we provide limited support as macrofungal community variation was determined more by substrate than forest age.

The comparatively higher taxonomic richness of leaf litter communities of managed relative to primary forests could be explained by compositional shifts to large genera which are more resistant to desiccation, such as *Marasmius*, *Marasmiellus*, *Tetrapyrgos* and *Micromphale,* typical of disturbed forests (Lodge & Cantrell 1995). For example, the diversity of hypocrealean taxa (Ascomycota microfungi) and wood decaying basidiomycetous diversity decreased in later successional stage. Similar works have showed that fungal species richness was negatively correlated to the age of forest stands in Costa Rica for wood decaying basidiomycetes (Chaverri & Vílchez 2006). In addition, Mesquita *et al*. (1998) observed in Amazonian secondary forest, a slow leaf litter decomposition and suggest that difference is not between pioneer *vs* primary forest plant species, but the overall environment between these forest and many important agents of decomposition may be absent from degraded secondary forest. In the other hand, was observed that total annual litter fall was similar in primary and secondary forests, and litter decomposition was similar in the primary and secondary forest at northeastern Brazilian Amazonia (Barlow *et al*. 2007). However, annual variation rate in decomposition of macrofungal biomass in wet tropical forests is demonstrably high and related to precipitation (Stark & Jordan 1978). So the

greater substrate diversity and lower turnover time could be promote leaf inhabiting fungal communities, in front other decomposer, especially during the rainy season.

IMPLICATIONS OF FOREST MANAGEMENT PRACTICES ON AMAZONIAN FRUITING MACROFUNGI.– Macrofungi play a crucial role in the biogeochemical cycles of forest's worldwide and potential impacts from anthropogenic disturbances on their communities, which may include forest management practices, may alter their structure in complex ways. Perhaps the greatest challenge to characterizing macrofungal communities is their high level of cryptic diversity as reflected by the fact that basidiome communities are not necessarily representative of the entire mycelial community (Porter *et al*. 2008). A comprehensive assessment of these communities demands implementation of either long term monitoring programs which implement multiple sampling protocols (Yamashita *et al*. 2015), the use of genetic screening techniques (Hartmann *et al*. 2014) or ideally a combination therein.

A majority of the taxa observed in this study were restricted to either one of the two forest types communities as singletons suggesting that spatial patterns of basidiome distributions are either larger than the spatial scale of the experimental design of the study area or truly reflect differences in substrate quality which, in turn influences either presence of mycelia and/or rates of basidiome expression. Nonetheless, overall estimated values of species richness demonstrate a negative synergistic effect of dry season and forest management on basidiome richness, and contrasting effects of these practices dependent on the guild in question.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version in this article:

TABLE S1. *Agaricales mushroom in Primary and Secondary forest at central Amazonia (abundance data)*.

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TABLE 1. *Number of taxa, number of individual and Fisher's alpha computed for Agaricales basidiome communities at secondary and primary forest according to substrate and sazonality in Central Amazonia*

.

	Taxa	**Individuals**	**α**
Trunk			
Primary	28	40	41.45
Secondary	23	34	31.22
Branch			
Primary	54	91	55.87
Secondary	54	90	57.00
Leaf			
Primary	84	178	62.13
Secondary	89	146	96.75
Soil			
Primary	53	63	157.61
Secondary	22	28	47.37
Total			
Primary	186	369	149.64
Secondary	160	298	140.69
Rainy Season			
Primary	177	329	156.11
Secondary	155	286	138.22
Dry Season			
Primary	29	43	39.07
Secondary	9	12	16.36

TABLE S1. *Agaricales mushroom collected during the project at permanent transects on primary and secondary forest in Central Amazonia*

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	**Secondary forest**	**Rainy**	**Dry**	**2012**	**2013**	**Soil**	**Trunk**	**Branch**	**Leaf**
Agaricus cf. *rufoaurantiacus* Heinem.		1	1			1	1			
Agaricus sp.1	1		1			1	1			
Arthromyces sp.1	1		1			1	1			
Caripia montagnei (Berk.) Kuntze	8	4	10	2	5	7		1	11	
Chaetocalathus sp.1	1		1			1			1	
Clitocybe sp.1	1		1		1		1			
Clitocybula sp.1	2		2			2	2			
Conocybe sp.1	1		1			1	1			
Crepidotus sp.1	1		1			1	1			
Crinipellis sp.1	1		1		1				1	
Crinipellis sp.2	1		1			1				1
Cryptomarasmius sp.1	1	3	4		4					4
Crysomphalina sp.1	1	1	2			2	2			
Cystolepiota sp.1		1	1		1		1			
Oudemansiella macracantha Singer	1		1		1			1		
Dactylosporina steffenii (Rick) Dörfelt	3		3		1	2		3		
Dennisiomyces sp.1	1		1		1		1			
Entoloma sp.1		2	2		2		2			
Entoloma sp.10		1	1			1				1
Entoloma sp.11		1	1			1				1
Entoloma sp.12		1	1			1	1			
Entoloma sp.13	1		1		1		1			
Entoloma sp.14		1	1			1	1			
Entoloma sp.15	1		1			1	1			
Entoloma sp.16		1	1		1		1			
Entoloma sp.17	1		1			1				1

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Gymnopus sp.14	5		5		3	2		2	3	
Gymnopus sp.15	3		3			3			2	1
Gymnopus sp.16		1	1		1					1
Gymnopus sp.17	4	7	9	2	4	7				11
Gymnopus sp.18	1	1	2			2				2
Gymnopus sp.19		1	1			1		1		
Gymnopus sp.2		2	2			2		1	1	
Gymnopus sp.20		5	5			5			4	1
Gymnopus sp.21		1	1			1			1	
Gymnopus sp.22	1			1	1				1	
Gymnopus sp.23		1	1		1					1
Gymnopus sp.24		1	1			1				1
Gymnopus sp.25	1		1			1				1
Gymnopus sp.26	1		1			1				1
Gymnopus sp.27		1	1			1				1
Gymnopus sp.28	2		2		1	1				2
Gymnopus sp.29	1		1		1					1
Gymnopus sp.3	1	1	2			2		1	1	
Gymnopus sp.30	2		2		1	1		1	1	
Gymnopus sp.4		4	4			4		2	2	
Gymnopus sp.5	4	5	9		1	8		1	3	5
Gymnopus sp.6		3	3		1	2				3
Gymnopus sp.7		1	1			1			1	
Gymnopus sp.8		1	1		1				1	
Gymnopus sp.9		2	2			2				2
Hemimycena cf. tortuosa	1		1		1				1	

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Hemiomyces aff. *neovelutipes*	1		1		1			1		
Hohenbuehelia sp.1		1	1		1			1		
Hohenbuehelia sp.2		1	1			1		1		
Hydropus sp.1	1		1			1	1			
Hydropus sp.2	2	2	4			4	1	2	1	
Hygroaster sp.1	1		1		1		1			
Hygrocybe sp.1		2	2			2	2			
Hygrocybe sp.2		2	2		2		2			
Hygrocybe trinitensis (Dennis) Pegler		2	2		1	1	2			
Hygrophorus sp.1	5		5		2	3	4			1
Inocephalus dragonosporus (Singer) T.J. Baroni & Largent		3	3		2	1	2			1
Lepiota sp.1		1	1			1		1		
Lepiota sp.2		1	1			1		1		
Lepiota sp.3		1	1			1	1			
Leucoagaricus sp.1	1			1	1		1			
Leucocoprinus brunneoluteus Capelari & Gimenes	1		1			1	1			
Macrocystidea sp.1	2	1	3			3	2			1
Marasmiellus "brown1"		1	1			1			1	
Marasmiellus "brown2"		1	1		1				1	
Marasmiellus "brown3"	2		2		1	1			2	
Marasmiellus "cream1"	1		1		1			1		
Marasmiellus "cream4"	1	1	2			2			1	1
Marasmiellus cubensis (Berk. & M.A. Curtis) Singer		2	1	1	1	1			2	
Marasmiellus volvatus Singer	2		2			2			2	
Marasmiellus "white1"	2		1	1	2				2	
Marasmiellus "white2"	1		1		1		1			

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Marasmiellus "white3"		1	1		1				1	
Marasmiellus "white4"	8	6	8	6	3	11			14	
Marasmiellus "white5"	1			1		1			1	
Marasmius atrorubens (Berk.) Mont.	1	1	1	1		2				2
Marasmius bellus Berk.	1			1		1				1
Marasmius "branched1"	1			1		1				1
Marasmius "branched2"		1	1			1				1
Marasmius "branched3"	2		2			2				2
Marasmius "branched4"		2	2			2				2
Marasmius "brown1"	1		1		1					1
Marasmius "brown2"	1		1		1					1
Marasmius "brown3"		2	2			2				2
Marasmius "brown4"		2	2			2				2
Marasmius "brown5"		1	1			1				1
Marasmius "brown6"		2	2		2					2
Marasmius "brown7"	2		2		2					2
Marasmius castellanoi Singer	8		7	1	4	4				8
Marasmius cf. *sanguirotalis*	1		1			1				1
Marasmius cf. *anomalus*		1	1			1				1
Marasmius cf. *nanorotalis*	2		1	1		2				2
*Marasmiellus "*cream2"		1	1			1			1	
Marasmius cladophyllus Berk.	2	4	5	1		6			1	5
Marasmius haematocephalus (Mont.) Fr.		1	1			1				1
Marasmius leoninus Berk.	2	2	4		1	3			4	
Marasmius "orange1"	1		1		1					1
Marasmius "orange10"		1	1			1				1

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Marasmius "orange11"		1	1			1				1
Marasmius "orange12"		5	5		1	4				5
Marasmius "orange13"	1		1		1				1	
Marasmius "orange14"	1	2	3		2	1				3
Marasmius "orange15"		4	4			4				4
Marasmius "orange16"	1		1		1					1
Marasmius "orange17"		1	1		1				1	
Marasmius "orange18"		1	1		1				1	
Marasmius "orange19"		1	1			1			1	
Marasmius "orange2"		2	2		2				1	1
Marasmius "orange20"	1	4	5			5			3	2
Marasmius "orange21"	1		1			1				1
Marasmius "orange22"		1	1			1				1
Marasmius "orange23"	2		2		1	1		1	1	
Marasmius "orange24"		1	1			1				1
Marasmius "orange3"		1	1			1				1
Marasmius "orange4"	2	1	2	1	2	1				3
Marasmius "orange5"	1	1	2		1	1				2
Marasmius "orange6"		1	1		1				1	
Marasmius "orange7"	2	3	5		2	3			3	2
Marasmius "orange8"	5	3	8		1	7			5	3
Marasmius "orange9"	1		1		1					1
Marasmius phaeus Berk. & M.A. Curtis	2	5	7		3	4		4	3	
Marasmius "pink"	1		1		1					1
Marasmius populiformis Berk.	1		1			1				1
Marasmius purpureobrunneolus Henn.		1	1			1			1	

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Marasmius "red1"	3	1	3	1	3	1				4
Marasmius "red10"		3	3		1	2				3
Marasmius "red11"	2	1	3			3			1	2
Marasmius "red12"	1		1			1				1
Marasmius "red13"	2		2		1	1				2
Marasmius "red14"	1		1			1			1	
Marasmius "red15"		1	1			1				1
Marasmius "red16"	1	1	2			2			1	1
Marasmius "red17"	2		2			2				2
Marasmius "red18"	2	1	3			3			1	2
Marasmius "red19"	1	1	2		1	1				2
Marasmius "red2"	1	1	2		2					2
Marasmius "red20"	1		1			1				1
Marasmius "red21"	1		1		1				1	
Marasmius "red22"		1	1		1					1
Marasmius "red23"	1	1	2		2					2
Marasmius "red3"		1	1		1					1
Marasmius "red4"	3	2	5		4	1				5
Marasmius "red5"		3	3		2	1			3	
Marasmius "red6"		1	1		1					1
Marasmius "red7"		2	2			2				2
Marasmius "red8"		2	2			2				2
Marasmius "red9"		1	1		1					1
Marasmius rotalis Berk. & Broome	5		4	1	2	3			1	4
Marasmius ruber Singer		1	1			1				1
Marasmius scleronematis Singer	1		1		1					1

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Marasmius sp. nov. *calvocystidiatus*	2		2		1	1				2
Marasmius tageticolor Berk.	3	1	3	1		4			1	3
Marasmius "white1"		1	1			1				1
Marasmius "white2"	1		1		1				1	
Micromphale sp.1	2	1	3			3		1	1	1
Morf1	1		1			1				1
Morf10	1		1			1		1		
Morf11		1	1			1	1			
Morf12	1		1		1		1			
Morf13	1		1			1	1			
Morf14		1	1			1		1		
Morf15	1		1			1		1		
Morf16	1		1			1	1			
Morf17	2		2			2	2			
Morf18	1		1		1				1	
Morf19	1		1		1		1			
Morf2		1	1			1				1
Morf20		1	1			1		1		
Morf21	2		2		2					2
Morf22	1		1			1	1			
Morf23	1		1		1					1
Morf24	1		1			1	1			
Morf25	1		1			1	1			
Morf26	1		1			1	1			
Morf27		1	1			1			1	
Morf28	1		1			1				1

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Morf29	1		1			1	1			
Morf3	1		1			1	1			
Morf30		1	1		1		1			
Morf31	1		1			1	1			
Morf32	1		1		1		1			
Morf33	1		1			1	1			
Morf34		1	1		1				1	
Morf35	1		1		1			1		
Morf36	1		1		1		1			
Morf37	2		2			2	2			
Morf38	1		1			1				1
Morf5	1		1			1	1			
Morf6	1	3	4		2	2	2			2
Morf7		1	1			1		1		
Morf8	1		1			1		1		
Morf9	1	1	2		2		2			
Mycena aff. *pura*		1	1		1					1
Mycena "brown1"	1	1	2			2				2
Mycena "brown2"	1	1	2			2				2
Mycena "brown3"	4	4	7	1	5	3				8
Mycena "brown4"	3		3		3					3
Mycena "brown5"		1	1			1				1
Mycena "brown6"	1	1	2			2				2
Mycena bulbosa (Cejp) Kühner	3	1	3	1	3	1				4
Mycena cf. *corynephora*	1		1		1					1
Mycena chloroxantha Singer	7	2	9		6	3			5	4

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Mycena "grey1"	1	4	5		3	2			2	3
Mycena "grey2"	2	1	3		3		1			2
Mycena "grey3"	1		1		1					1
Mycena "grey4"	1		1			1		1		
Mycena ixoxantha Singer	17	4	17	4	7	14			2	19
Mycena longicrinita Singer	3		2	1	2	1				3
Mycena margarita (Murrill) Murrill		1	1			1		1		
Mycena "pink1"	6	2	8		7	1			1	7
Mycena "pink2"		2	2			2				2
Mycena "red2"	1		1		1					1
Mycena spinosissima (Singer) Desjardin	11	16	21	6	18	9		1	22	4
Mycena "white1"	3		3		3					3
Mycena "white10"	1	2	3		3					3
Mycena "white11"	1		1			1				1
Mycena "white12"	1		1		1				1	
Mycena "white13"		1	1			1				1
Mycena "white14"	1		1		1				1	
Mycena "white15"		1	1		1				1	
Mycena "white2"	3		3		3					3
Mycena "white3"	3	2	5		4	1				5
Mycena "white4"		6	6		1	5		5	1	
Mycena "white5"	1	1		2	2					2
Mycena "white6"	1		1		1					1
Mycena "white7"	3		3		2	1	1	1		1
Mycena "white8"	3		3		2	1		3		
Mycena "white9"	1		1		1				1	

Table S1 (*continued*).

Taxa	Forest stage		Season		Year		Substrate			
	Primary forest	Secondary forest	Rainy	Dry	2012	2013	Soil	Trunk	Branch	Leaf
Mycena "yellow1"	21	4	21	4	17	8				25
Mycena "yellow2"	5		3	2	1	4				5
Omphalina sp.1	1		1		1			1		
Omphalina sp.2		1	1			1		1		
Pleurotoid morf1		1	1			1			1	
Pleurotoid morf2		1	1			1			1	
Pleurotoid morf3	1		1			1			1	
Pleurotoid morf4		1	1			1			1	
Pluteus sp.1	1		1		1			1		
Pluteus sp.2	10	3	13		7	6		12	1	
Pouzarella sp.1	1		1			1	1			
Psathyrella sp.1	6		5	1	1	5	2	1	1	2
Pseudobaepora sp.1	1		1		1		1			
Rhodocollybia sp.1	3		3		2	1		3		
Rhodocollybia sp.2	1		1		1		1			
Rhodocollybia sp.3		1	1		1		1			
Rhodocollybia sp.4	1			1	1		1			
Rhodocollybia sp.5	3		3			3	2			1
Rhodocybe sp.1	1			1		1				1
Tetrapyrgos longicystidiata A.H. Honan, Desjardin & T.J. Baroni	1	2	3		1	2		1	1	1
Tetrapyrgos sp. nov. *albonigripes*	2	1	3			3			2	1
Tetrapyrgos sp. nov. *brunneilucida*	2	2	4			4		1	2	1
Tetrapyrgos sp. nov. *pileobrunnea*	1	1	2			2			2	
Tetrapyrgos sp. nov. *pseudonigripes*		1	1		1				1	
Tetrapyrgos sp.1	1		1			1			1	
Tetrapyrgos sp.2		1	1		1					1
Tubaria sp.1	1		1		1		1			

FIGURE LEGENDS

Proposed Supplementary Figures

FIGURE S1. Localization of the collection site at Estação experimental de manejo florestal- ZF2. A- Brazil map, green cicle is Manaus area at Amazonas state; B- showing Manaus (pink area), collection area is between numbers 1 and 2; C- transects at BIONTE´s Project site. Circles are transects on the old forest, on the left of Block I and on the right of Block II; squares are transects on the secondary forest after clear-cutting. (C-Image modified from INPA 1992).

FIGURE S2. Principal Coordinates Analysis plots showing compositional separation of fungal communities in management area at Central Amazonia according to forest and substrate. (A) Based on abundance data; (B) Based on presence and absence data.

Proposed Figures for Text

FIGURE 1. Observed taxa accumulation curves of primary and secondary forest at different substrate and season in management area at Central Amazonia. (A) trunk; (B) branch; (C) leaf; (D) soil; (E) rainy season, and (F) dry season. Agaricales based on the mean of 1000 randomized orderings of sample number.

FIGURE 2. Principal Coordinates Analysis plots showing fungal communities according to forest, substrate and year collection in management area at Central Amazonia.

FIGURE 3. Sample sites and macrofungal diversity and substrate. (A) and (B) Primary forest; (C) and (D) Secondary forest; (E) *Leucocoprinus brunneoluteus* on soil; (F) *Marasmius bellus* on leaf; (G) *Marasmius tageticolor* on leaf; (H) *Mycena spinosissima* on branch; (I) *Marasmius phaeus* on trunk. Scale bars: E=20; H=10; F, G, I=5 mm.

FIGURE S1

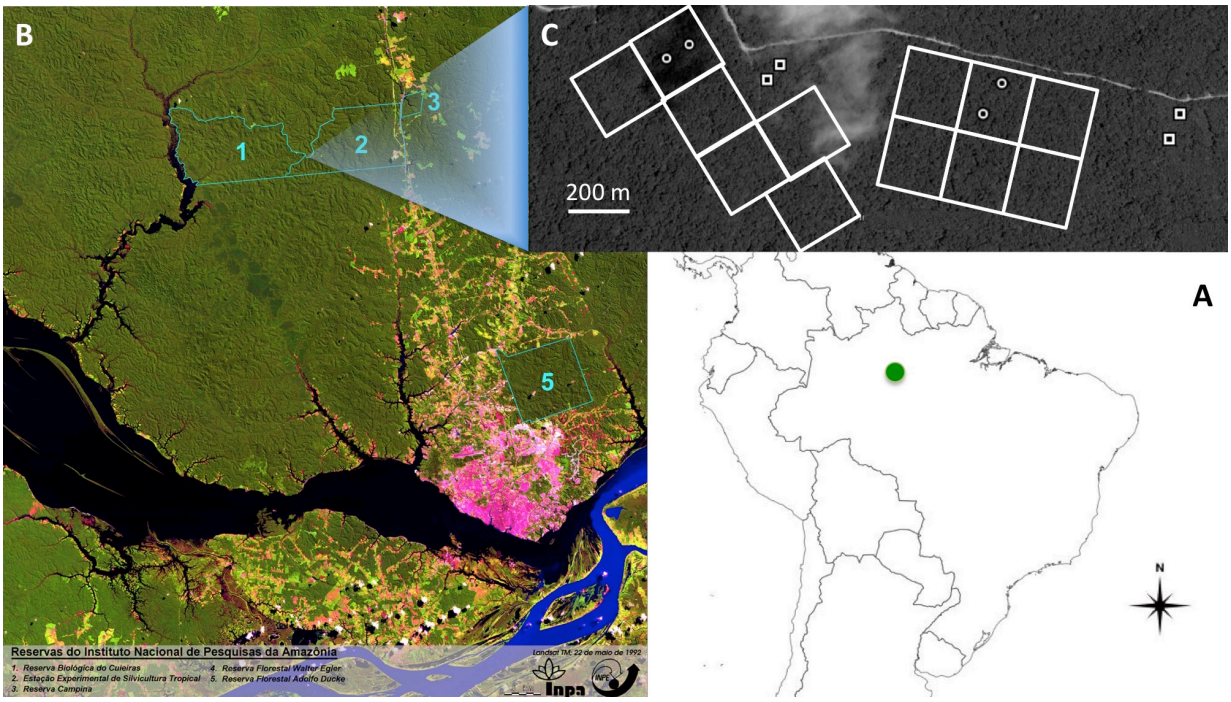

FIGURE S2

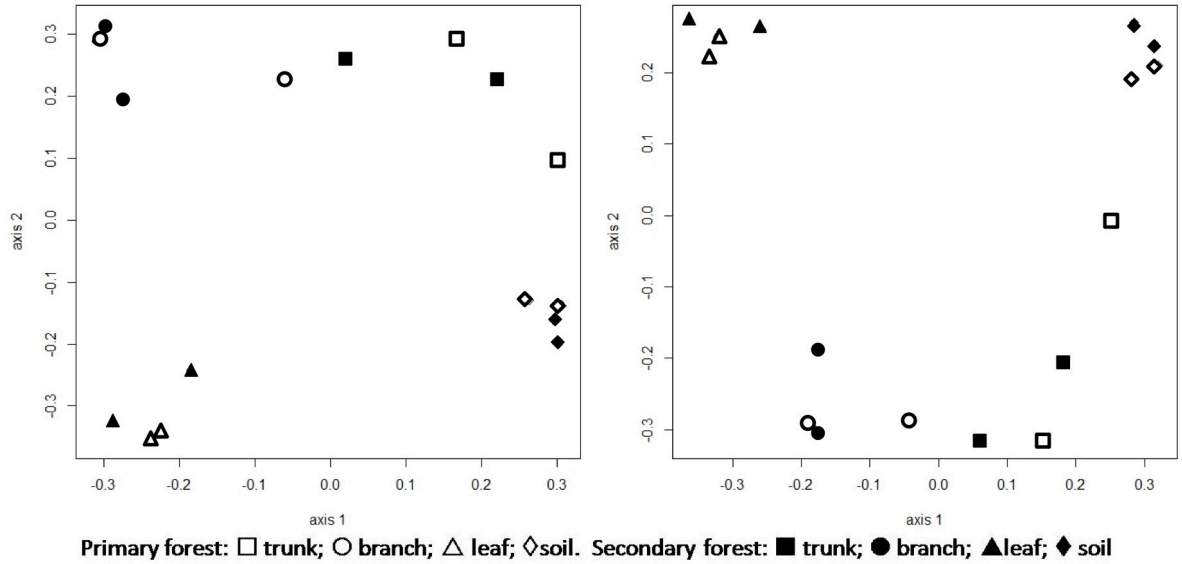

FIGURE 1

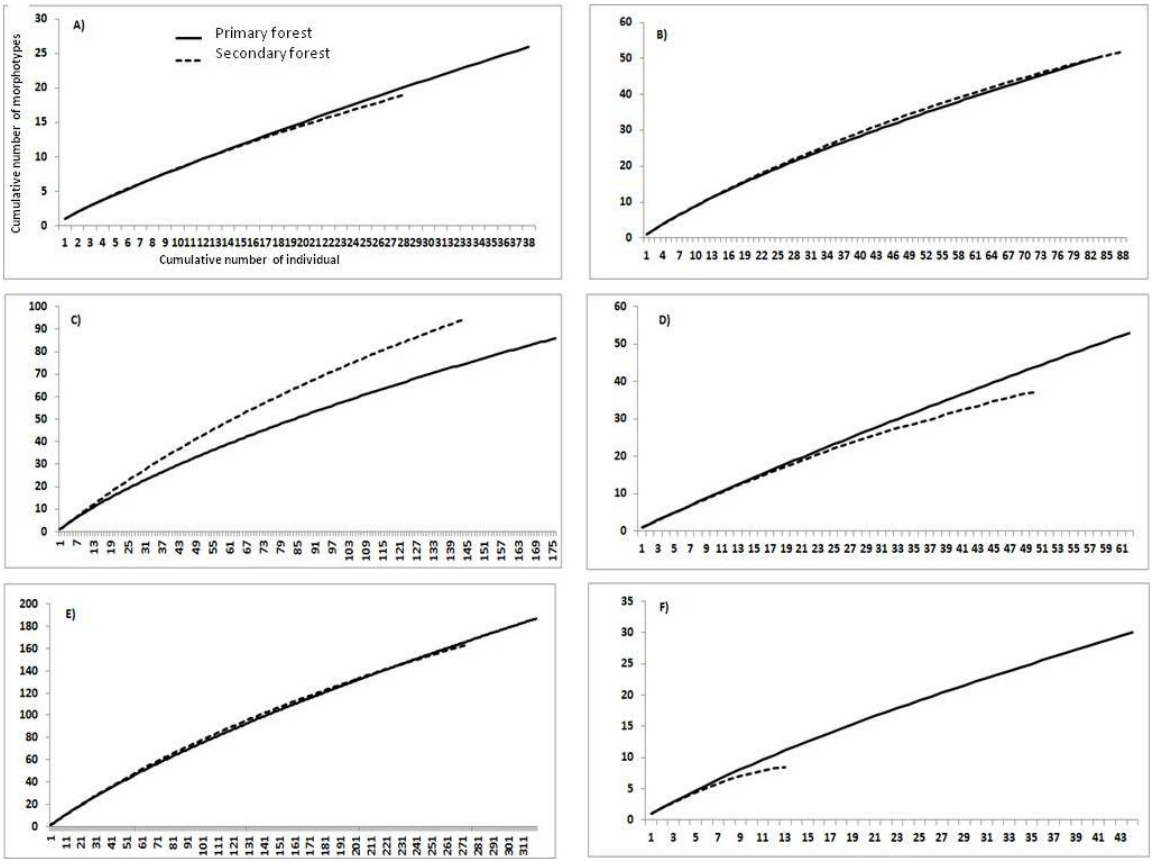
A)
Primary forest
Secondary forest
Cumulative number of morphotypes
Cumulative number of individual
B)
C)
D)
E)
F)

FIGURE 2

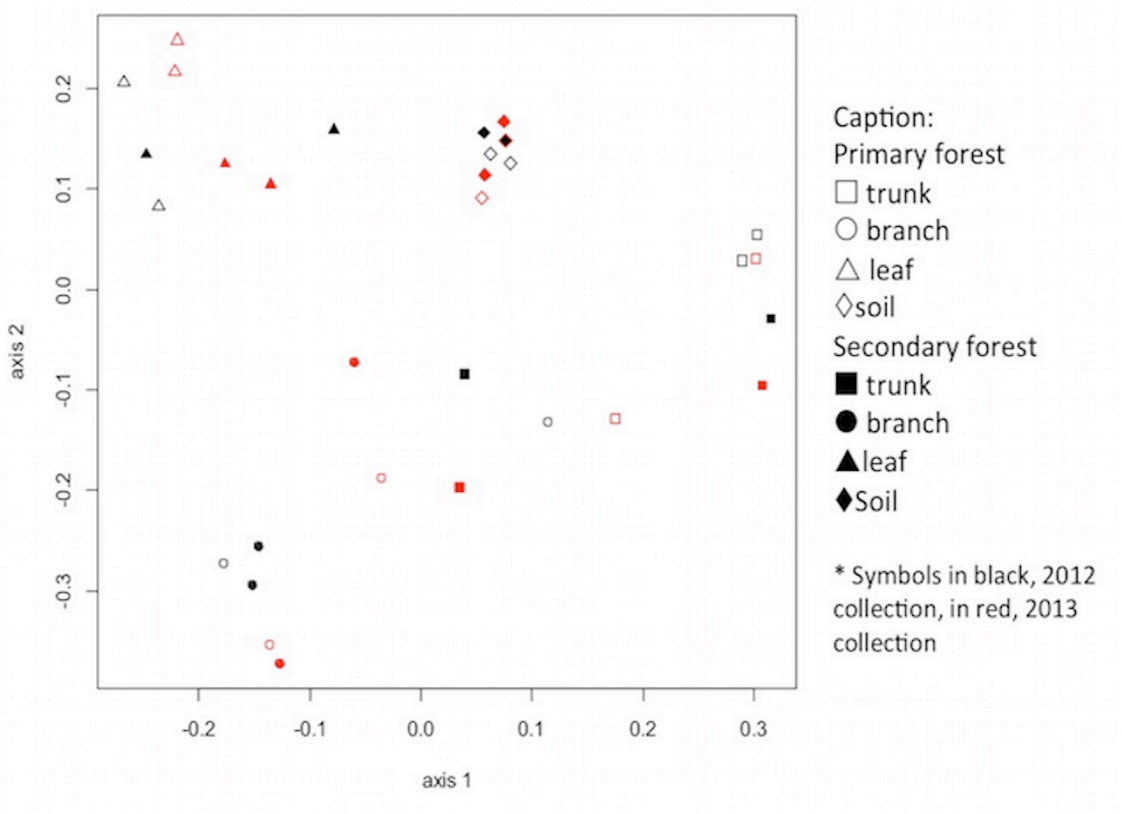
axis 2
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
axis 1
Caption:
Primary forest
trunk
branch
leaf
soil
Secondary forest
trunk
branch
leaf
Soil
* Symbols in black, 2012 collection, in red, 2013 collection

FIGURE 3

Capítulo 2

Komura, D.L; Moncalvo, J. M.; Margaritescu, S.; Zartman, C. E. Six new species of *Tetrapyrgos* from the Brazilian Amazon

Manuscrito submetido para Mycologia (aceito, em processo de revisão)

Short title for running head: Six new species of *Tetrapyrgos*

Six new species of *Tetrapyrgos* from the Brazilian Amazon

Dirce Leimi Komura[1]

Instituto Nacional de Pesquisas da Amazônia, Botany, Botany Department , C.P. 478, 69067-375 Manaus, Amazonas, Brazil

Jean-Marc Moncalvo

Department of Natural History, Royal Ontario Museum & Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

Simona Margaritescu

Department of Natural History, Royal Ontario Museum, Toronto, Canada

Charles Eugene Zartman

Instituto Nacional de Pesquisas da Amazônia, Botany Department, 69067-375 Manaus, Amazonas, Brazil

Abstract: *Tetrapyrgos* (Marasmiaceae, Agaricales, Basidiomycota) is characterized by a well-defined central or eccentric stipe, a non- or very weakly gelatinous pileipellis and pileus trama, pileocystidia with *Rameales*-like cells, and distinctly tetrahedral basidiospores. Members of this genus are little whitish mushrooms commonly found on dead leaves and wood debris on the floor of tropical forests, but their taxonomic diversity is still poorly documented. Recent studies suggest its monophyly with *Campanella* as sister group within the Marasmiaceae. To date, eight species are recognized within the genus *Tetrapyrgos*. Here we describe six new species from the Brazilian Amazon, and provide a key for the described Amazonian species. Finally, on the basis of available ITS sequences, this study also points out the existence of several additional phylogenetically distinct species that still need to be properly morphologically described, and named.

Key words**:** Agaricales, Fungi, ITS, Marasmiaceae, Taxonomy, Tropical Forests

INTRODUCTION

Tetrapyrgos belongs to Marasmiaceae Roze ex Kühner, which is composed of 54 genera (+ 30 syn.) and around 1590 spp. (Matheny et al. 2006, Kirk et al. 2008). Recent works based on molecular data shows great evidences that *Tetrapyrgos* Horak, *Campanella*, *Marasmius* Fr., *Chaetocalathus* Singer, *Crinipellis* Pat. and *Moniliophthora* H.C. Evans, Stalpers, Samson & Benny are a natural group in Marasmiaceae (Moncalvo et al. 2002, Matheny et al. 2006). Those small white spore mushrooms are almost cosmopolitan, but are more diverse on the tropical forest, and commonly found on leaves and wood debris in the floor (Singer 1986).

In spite of their presence in lowland tropical forests worldwide, the taxonomy of this family remains poorly understood. Such complication is best illustrated in the taxonomic history of *Tetrapyrgos*, a name proposed by Horak (1986) in order to accommodate for the fact that *Pterospora* Métrod (Métrod 1949) holds priority as the name of an angiosperm taxon in the Ericaceae. The type species of *Tetrapyrgos*, *T. atrocyanea* (Métrod) E. Horak, is characterized by tetrahedral spores and a pileocutis with Rameales-structure. Horak (1983, 1986) recognized 15 species in the genus, including taxa formerly classified in *Campanella* (Singer 1945, 1955, 1969, 1973, 1975a, b; Redhead 1974), *Marasmiellus* (Singer 1946, 1973; Reid 1966) or *Marasmius* (Berkeley and Broome, 1873). An additional species was later described by Petersen and Gordon (1994), bringing to 16 the number of species currently accepted in *Tetrapyrgos* in both Index Fungorum (http://www.indexfungorum.org) and MycoBank (http://www.mycobank.org).

However, more recently, Honan (2007) and Honan et al. 2015 revisited the circumscription of *Tetrapyrgos* by using both morphology and ITS-rDNA sequence data and reduced the number of recognized taxa in the genus to six: *T. atrocyanea* (Métrod) E. Horak, *T. nigripes* (Fr.) E. Horak, *T. reducta* (Singer) E. Horak, *T. subcinerea* (Berk. & Broome) E. Horak, *T. subdendrophora* (Redhead) E. Horak, and *T. tropicalis* R.H. Petersen & S.A. Gordon, while suggesting to retain the other *Tetrapyrgos* species *sensu* Horak (1986) in

Campanella Henn and two more new species, *T. longicystidiata* A. H. Honan, Desjardin & Baroni and *T. parvispora* A.H. Honan & Desjardin (Honan et al 2015).

Molecular studies, which have evaluated the phylogenetic relationships of *Tetrapyrgos* and *Campanella* in the context of the Agaricales, consistently show the monophyly of these two genera within the Marasmiaceae (Moncalvo et al. 2002, Wilson & Desjardin 2005, Matheny et al. 2006). However, such studies are based on relatively limited taxon sampling that also did not include type species of these genera.

Honan (2007) indicated the following characters for morphological distinction between these two genera: *Tetrapyrgos* basidiomes have a well-defined central or eccentric stipe (vs. sessile or with a pseudostipe in *Campanella*), non- or very weakly gelatinous pileipellis and pileus trama (vs. clearly gelatinous), and distinctly tetrahedral basidiospores (vs. ellipsoid or bulging on one side).

Here we follow the morphological genus concept of *Tetrapyrgos* proposed in Honan (2007) to describe six new species from the Brazilian Amazon, that are also supported by the Internal Transcribed Spacer gene of the ribosomal DNA array (ITS).

MATERIAL AND METHODS

Fieldwork.— Specimens were collected during field expeditions in 2012-2015 at four sites in the Brazilian Amazon: (1) The Estação Experimental de Manejo Florestal do INPA (ZF-2) (02°37’ S, 60°09’ W), about 80 km north of Manaus, the state capital of Amazonas; (2) The Reserva de Desenvolvimento Sustentável do Tupé (3° 07' S, 60° 18' W), 25 km west of Manaus on the banks of the Rio Negro; (3) Cachoeira da Iracema, Presidente Figueiredo (1° 59' S, 60° 03' W), 100 km north of Manaus; and (4) The lower Aracá River (0° 07' S ,63° 19' W), approximately 400 km northwest of Manaus.

Morphological descriptions.— Freshly collected specimens were described macroscopically, and digital images were taken. We used the color code based on Color Picker chart color (http://www.colorpicker.com). Collections were dried at 40-50 °C with the use of an electric dehydrator (A. & J. Stöckli) or in silicagel for subsequent microscopical examination and herbarium preservation. Microscopical observations were carried out by rehydrating sections of dried materials in 70% ethanol and subsequent mounting in 5% KOH or Congo Red solution (Singer 1986), and illustrated with the use of a drawing tube. Basidiospores measurements (length ×

width) were summarized by using the follow calculation formulas: X_m, the arithmetic mean (± standard deviation) of length × width; and Q_m, the mean (± standard deviation) of length and width of basidiospores. Lamellae spacing was estimated by tallying the number of lamellae that reach from the stipe to the pileus margin (*L*) as well as the number of series of lamellulae (*l*) among the lamellae (Desjardin et al. 1991; Honan 2007). The dried collections were deposited in the INPA herbarium with duplicates in the Royal Ontario Museum Fungarium (TRTC).

ITS sequences production and analysis.— DNA isolation, PCR amplification, sequencing and editing of the ITS region followed Dentinger et al. (2010) and were carried out at Laboratory of Molecular Sistematic from Royal Ontario Museum, Toronto, CA. Sequences showing >90% similarity to the newly produced *Tetrapyrgos* sequences were retrieved from BLAST (Altschul et al. 1990) searches in the NCBI database (GenBank), and alignment were performed in MEGA vs. 5.05 using MUSCLE (Edgar, 2004) A preliminary analysis was conducted in MEGA version 6 (Tamura et al. 2013) using Maximum-Likelihood (ML) and default parameters. From this preliminary analysis (data not shown) we selected 70 ITS sequences (Table I) that were deemed to be relevant for this study, *Marasmius rotula* was used as outgroup as in Honan et al. 2015. ML settings for the final analysis were determined in jModeltest 2.1.7 (Darriba et al. 2012). Bootstrap support (BS) for branches was estimated from 1,000 replications. Bayesian analyses were run in MrBayes version 3.2.1 (Ronquist et al., 2012). A best-fit nucleotide substitution model was first selected via the Akaike information criterion (AIC), as implemented in MrModelTest version 2.1.7. In two separate runs of a Metropolis-coupled Markov Chain Monte Carlo (MCMC) permutation of parameters, six simultaneous chains were initiated with a random tree for 10 million generations through the phylogenetic tree space, sampling one tree at each 500th generation. Non-autocorrelated samples at the stationary phase were summarized in a Bayesian majority rule consensus tree at 50% after a burn-in of 25% and was based on a total of 15002 samples from 2 runs. Clade frequencies or posterior probabilities (PP) represent support measures (Huelsenbeck et al., 2002). This analysis was run through the CIPRES Science Gateway v. 3.3 (Miller et al. 2010; http://www.phylo.org).

RESULTS

Fieldwork resulted in 25 collections that correspond to *Tetrapyrgos*, from which we obtained 21 ITS sequences. Nineteen basidiomes were identified to the species level; two were unique specimens (*Tetrapyrgos* sp.1 and sp.2) of poor quality, from which we could not derived enough morphological information to infer as a new species. So, based on combined evidence from ITS sequences and morphological characters, we identified seven species: one

is *T. longicystidiata*, and the other six are proposed to be new to science. We named them *T. albonigripes*, *T. brevipileocystidiata*, *T. brunneilucida*, *T. cystidiacrassa*, *T. pileobrunnea*, and *T. pseudonigripes*.

ITS sequence analyses.— The final data matrix consisted of 70 sequences (Table I) that unambiguously aligned in 569 positions. The AIC criterion in jModeltest indicated that the HKY-I-G model best suits the dataset, with the following parameters estimated in MEGA (I=44.92%, G=0.2850). The resulting tree (-ln=2529.1024) is depicted in FIG1, with both Posterior probalility in Bayesian analysis (PP) and support from Bayesian analysis and Maximun Likelihood bootstrap support (BS). The analysis supports monophyly of *T. longicystidiata* (0.99 PP; 99% BS), *T. cystideocrassa* (0.98 PP; 99% BS), *T. pileobrunea* (99% BS, 2% IV; N=2), *T. pseudonigripes* (0.83 PP; 99% BS), *T. nigripes* from USA (0.98 PP; 99% BS), *T. brevipileocystidiata* (0.99 PP; 89% BS), *T. bruneilucida* (1.0 PP; 99% BS), *T. parvispora* (0.99 PP; 99% BS), *T. albonigripes* (0.70 PP; 100% BS), *T.* aff. *nigripes*) and *T. subdendrophora* (1.0 PP; 100% BS). Genbank collections labeled *T. subcinerea* split into two distinctive subclades (1.0 PP; 98% BS and another with support < 0.7 PP and 70% BS), as do collections labeled *T. subdendrophora*. Genbank collections labeled *T. nigripes* and *T. aff. nigripes* are polyphyletic. Two collections stand alone (AM15316, *Tetrapyrgos* sp1, and DLK1970, *Tetrapyrgos* sp2). Two environmental sequences from Guyana (JN890461, JN890458) cluster within the *Tetrapyrgos* clade. Overall, basal relationships are poorly resolved, with the exception of a strong sister group relationship between *T. longicystidiata* and *T. cystideocrassa* (0.98 PP; 96% BS). *Marasmius* species used as out group formed a long branch and samples from *Campanella* and *Marasmiellus* grouped in distint clade, but without support.

TAXONOMY

Tetrapyrgos albonigripes D.L. Komura & J-M. Moncalvo sp. nov. FIG. 2

MycoBank MBXXXX

Typification: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 25 Apr 2013, *D.L.Komura & P.A. Pereira DLK1360* (**holotype** INPA259599). GenBank accession: ITS KT287081.

Etymology: This species is similar to *T. nigripes* but the dried specimens retain the pale-cream color of the pileus.

Diagnosis: The macro and micromorphology are very similar to those of *T. nigripes*, with the exception of a thicker context and a pale-cream pileus that retains its color upon drying. The basidiospores size is similar, our specimen reaches 7.6–10.7 × 6.6–8.6 μm while *T. nigripes* (Wong88) has 9.6–11.2 × 6–8 μm. The number of lamellae is more numerous (*L*= 16–17 *versus* 9–13) and is not intervenose.

Description: Pileus 8–15 mm diam; convex to plan; uplift, wavy margin, striated, scurfy, dry, opaque, pruinose; white-grey (B5BD8A), cream (FCF9CF) in dried material. *Lamellae* adnate, *L*= 16–17, *l*= 2–3, white-greyish (B5BD8A), edge granular under lens; *Stipe* 10–16 × 0.5–1.5 mm, central, cylindrical, thin, horny, flexible, hollow; dark grey (757575) with some white covering; narrow mycelial pad, greyish (82827F).

Basidiospores 7.6–10.7 × 6.6–8.6 μm [X_m= 9.1 ±0.9 × 7.6 ±0.5 μm; *Q*= 1.04–1.36, Q_m= 1.2 ±0.1; n= 20], tetrahedral, smooth, thin-walled, hyaline, inamyloid. *Basidia* 27–29 × 9–10 μm, clavate, 4–sterigmate. *Basidioles* 27–28 × 7–8 μm, clavate. *Pleurocystidia* absent. *Cheilocystidia* abundant, main body 45–60 × 7–8 μm, elongate, irregularly branched, diverticulate, hyaline, thin-walled; diverticula, 2–4 × 0.5–2 μm, knob-like, most bulbous apex. *Lamellar trama* interwoven, cells 4–5 μm diam, hyaline. *Pileipellis* a *Rameales*-structure, subgelatinous. *Pileocystidia* main body 20–50 × 3–10 μm, diverticulate overall, rod-like, most cells irregular usually with a bulbous to bulboid apex, forked, hyaline, thin-walled; diverticula

1–10 × 0.5–5 µm, versiform, obtuse, elongated, clavate. *Pileus trama* interwoven, cells 3–5 µm diam, hyaline. *Stipitipellis* trama with parallel cells, cylindrical, brownish, thick-walled, around 5 µm diam. *Caulocystidia* main body 2–70 × 5–6 µm, most elongate, diverticulate, bulbous, bifurcate, branched apex, hyaline, some brown and thick-walled; diverticula 0.5–3 × 1–3 µm, obtuse, acute, pyriform, hyaline. Covering along all the stipe, more concentrated upward to pileus, showins a pruinous aspect to the stipe surface.

Clamp connections present in all tissues examined.

Habit and Habitat: Solitary on dicotyledonous leaves at leaf litter in Amazonian terra-firme forest.

Distribution: Brazil, Amazonas

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 21 May 2013, *O.F. Menezes & D.L. Komura, DLK1453* (INPA259603); 21 Jun 2013, *D.L.Komura & M.R.Pereira; D.S.Ferreira; L.S.Bento DLK1699* (INPA259605).

Comments: This species resembles the morphologically highly variable *Tetrapyrgos nigripes sensu* Horak and *sensu* Singer (as *Marasmiellus nigripes*) by having a whitish and pruinose pileus, diverticulate and bulbous apex cystidia. The macro-morphology of *T. nigripes* as described to specimen Wong888 from Hawaii (Desjardin et al. 1992) and TOR89 from British Virgin Islands (Honan 2007; Honan et al. 2015) is very similar, but the ITS analysis have placed our specimens in a distinct clade. *Tetrapyrgos albonigripes* has the double of number of lamella (*L*= 16–17) when compare with *T. pseudonigripes* (*L*= 8), also has a thicker context and when dried keeps a pileus with cream pale color, differing from *T. pseudonigripes* in becoming relatively more fragile and brown-colored when dry.

Tetrapyrgos brevipileocystidiata D.L. Komura & J-M. Moncalvo sp. nov. FIG. **3**

MycoBank MBXXXX

Typification: BRAZIL, AMAZONAS, Presidente Figueiredo, Cachoeira da Iracema, 31 May 2013, *D.L.Komura; T.H.G. Oliveira & A. Melo DLK1602* (**holotype** INPA259604). GenBank accession: ITS KT287088.

Etymology. Based on reduced length of the pileocystidia relative to other sister species.

Diagnosis: This species is characterized by strong umbilicate pileus which are white turning brownish with age, and short pileocystidia up to 30 µm long.

Description: Pileus 15–22 mm diam., pure white, turning cream to brownish (C79656) with age, center depressed, striated, smooth. *Lamellae* cream, intervenose, membranaceous, adnate, some forked; *L*= 12–17, *l*= 1–2 series; white, pale (DECAAF) with age; *Stipe* 8–10 × 1–2 mm; central, cylindrical, thin, thicker at apex, circular to depressed, horny, flexible, hollow; black with white hairs, turning white to apex with granular white covering; mycelial pad inconspicuous.

Basidiospores 8–16 × 6–15 µm [X_m= 12.4 ±2.8 × 10.6 ±2.8 µm; *Q*= 1–1.5, Q_m= 1.2 ±0.2; n=20]; tetrahedral, smooth, thin-walled, hyaline, inamyloid; *Basidia* 32–35 × 10–15 µm, clavate, 4-sterigmate. *Basidioles*, 35–40 × 6–7 µm, clavate. *Pleurocystidia* absent. *Cheilocystidia* main body 20–40 × 8–10 µm, elongated cells, irregularly branched, diverticulate, hyaline, thin-walled; diverticula, 1–6 × 0.5–5 µm, elongate, bifurcated, knob-like, bulboid. *Lamellar trama* regular, interwoven cells 4–5 µm diam, hyaline. *Pileocystidia* main body 19–30 × 9–12 µm, diverticulate overall, rod-like, most irregular usually with a bulboid apex, hyaline, thin-walled, diverticula 1–6 × 0.5–5 µm. *Pileipellis* irregular. *Context* irregular with interwoven cells, hyaline, 3–5 µm diam. *Stipitipellis* trama with hyphae 910 µm, parallel, cylindrical, brownish-golden, thick-walled and skeletal hyphae, 3–4 µm diam,

brownish-golden, thick-walled. *Caulocystidia* 20–100 × 4–5 µm, diverticulate, branched, irregular, most with bulbous apex, hyaline, some very long and branched at apex; diverticula 0.5–10 × 0.5–5 µm, knob-like, some acute, other obtuse, bulboid. *Clamp connections* present.

Habit and Habitat: Solitary on branches and leaves on the leaf litter of understory trees in Amazonian Campinarana forest.

Distribution: Brazil, Amazonas

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, RDS Tupé, Agrovila, 24 Mar 2013, *D.L.Komura & Cardoso, D. DLK1065* (INPA270737).

Comments: This species is characterized by strong umbilicate pileus that is white turning brownish with age, anastomosed lamellae and shorter pileocystidia, up to 30 µm long. Desjardin (1991) and Honan et al. (2015) described an umbilicate form to *Tetrapyrgos nigripes*, but our specimen does not have a pruinose pileus, neither turning grey spot with age. The change color in some *Tetrapyrgos* species is used as distinguish characteristic between species (Honnan et al. 2015). Pileocystidia is very distinguishable, not described for other species, once the usual pileocystidia in *Tetrapyrgos* is diverticulate and with bulbous apex, but this one is shorter, up to 30 µm long and pyriform with diverticula on the top of the cells.

Tetrapyrgos brunneilucida D.L. Komura & J-M. Moncalvo sp. nov. FIG. 4

MycoBank. MBXXXX

Typification: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF2), 25 Apr 2013, *D.L.Komura & P.A. Pereira DLK1339* (**holotype** INPA259594). GenBank accession: ITS KT287090.

Etymology. Based on the brownish and shiny pileus.

Diagnosis: This specimen is characterized by having a convex, strongly sulcate and slightly shiny pileus; the disc at the base of the stipe turns pale brown and pruinose with age. Pileocystidia are mainly irregularly branched.

Description: Pileus 10–12 mm diam, plane to slightly or broadly convex, sulcate, smooth, slightly shiny, margin incurved; brown (4D3218) turning pale (E6DDCA) to margin, cream (E0D9B6) when dried. *Lamellae* adnate, slightly intervenose; *L*= 11–15, *l*= 1–3 series; cream (F5EECB) turning pale brown (D6B487) with age. *Stipe* 10–15 × 1 mm, central, cylindrical thin, circular, horny, flexible, hollow; black turning white to apex with granular white covering; inconspicuous mycelial pad, dark grey (423F3B) pale brown (D6B487) and pruinose with age.

Basidiospores 10–15 × 8–12 µm [X_m= 11.7 ±1.4 × 9.9 ±1.0 µm; *Q*= 1–1.4, Q_m= 1.2 ±0.1; n=20], tetrahedral, smooth, thin-walled, hyaline, inamyloid. *Basidia* 50 × 14 µm, clavate, 4-sterigmate. *Basidioles* 35–40 × 10–12 µm, clavate, some fusiform. *Pleurocystidia* absent. *Cheilocystidia* main body 40–60 × 9–14 µm, elongate cells, diverticulate, irregular, some somewhat pyriform to clavarioid at apex, hyaline, thin-walled; diverticula, 1–8 × 0.5–4 µm, elongate, bifurcated, knob-like, bulboid, digitiform. *Lamellar trama* regular, interwoven cells 4–5 µm diam, hyaline. *Pileocystidia* main body 30–40 × 5–6 µm, branched, some bifurcated, irregular, lobate, hyaline, thin-walled. *Pileipellis* regular, somewhat gelatinized, *Context* irregular with interwoven cells, hyaline, 7–9 µm diam. *Stipitipellis* trama cells around 5 µm diam with parallel, cylindrical, brownish golden, thick-walled, clamped, some cells; *Caulocystidia* 50–100 × 4–5 µm, somewhat similar to cells of cheilocystidia but elongate and bulbous apex, diverticulate, lobate, hyaline. *Clamp connections* present.

Habit and Habitat: Solitary to gregarious on branches of dicotyledonous plants at leaf litter on Amazonian terra firme forest.

Distribution: Brazil, Amazonas

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 24 Apr 2013, *D.L.Komura & P.A. Pereira DLK1213* (INPA259601).

Comments: This specimen is characterized by convex, strongly sulcate, and slightly shiny pileus. The disc of the stipe turns pale brown, and pruinose with age. Pileocystidia are mainly

irregular and branched, somewhat similar to the ones described in *T. tropicalis* R.H. Petersen & S.A. Gordon (1994) from Puerto Rico, Caribbean USA. The basidia and basidioles are branched in both species. However, the macro morphology is very different; *T. tropicalis* has conchate to reniform, pruinose, white pileus and distant lamellae.

Tetrapyrgos cystidiacrassa D.L. Komura & J-M. Moncalvo sp. nov. FIG. 5

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Typification: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 19 Apr 2012, *D.L.Komura & J.M.Moncalvo; C.E.Zartman DLK336* (**holotype** INPA259606). GenBank accession: ITS KT287092.

Etymology: Based on pileocystidia with characteristically brown and thick-walled cells.

Diagnosis: This species has the most variable type of the pileocystidia as some cells are thick-walled and brownish whereas others are thin excrescences covering these cells.

Description: Pileus 15 mm diam, broadly convex, suede-like, greyish-brown with brown dots under lens (6E6B5A) to olivaceus, opaque. *Lamellae* adnate to decurrent, slightly intervenose; *L*= 11, *l*= 1–2; white greyish (C2C1B2). *Stipe* 13 × 1 mm, central, cylindrical, thin, thicker at apex, circular, horny, flexible, hollow; entirely black with coiled, white covering; narrow mycelial pad, grey (545452).

Basidiospores 10–17 × 9–15 µm [X_m= 13.5 ±2.0 × 12 ±2.0 µm; *Q*= 0.86–1.50, Q_m= 1.33 ±0.1; n=20], tetrahedral, smooth, thin-walled, hyaline, inamyloid. *Basidia*, 32–37 × 6–7 µm, 4-sterigmate, clavate. *Basidioles* 35–42 × 10–11 µm, clavate, some elongate. *Pleurocystidia* absent. *Cheilocystidia* main body 22–60 × 6–10 µm, branched, diverticulate, irregular, hyaline, thin-walled; diverticula, 1–3× 0.5–2µm, bifurcated, knob-like. *Lamellar trama* regular, interwoven cells 4–5 µm diam, hyaline. *Pileocystidia* main body 35–60 × 7–30 µm, diverticulate overall, many clavate with excrescences, with or without acute diverticula;

frequently somewhat Rameales type cells, thick-walled, brownish others elongate, lobate, diverticulate, irregular, thin-walled, hyaline. *Pileipellis*, irregular, brownish. *Context* irregular with interwoven cells, hyaline, 4–5 µm diam. *Stipitipellis* trama with parallel hyphae, cylindrical, brownish-golden, thick-walled 4–10 µm diam; some cells, 2–3 µm diam, setaceous, brownish, thick-walled. *Caulocystidia* main body 40–70 × 7–12 µm, elongate, diverticulate, lobate, hyaline cells; diverticula 0.5–5 × 0.5–2 µm, obtuse, bifurcate, hyaline; lobes 5–10 × 3– 5 µm, hyaline, thin-walled. *Clamp connections* present in all tissues.

Habit and Habitat: Solitary on dead branches of dicotyledonous plants at leaf litter in Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, Jardim Botânico Adolpho Ducke, 25 Apr 2012, *D.L.Komura & J.M.Moncalvo DLK374* (INPA259607).

Comments: This species has variable type of the pileocystidia, a very uncommon structure in *Tetrapyrgos*, cells with thick-walled and brownish and some cells covered with thin excrescences. Metuloid plerocystidia were described by Singer in *T. simulans* (Pat.) E. Horak from Hawaii, but this species has more characteristics related to *Campanella*, such as lack of stipe and tetrapodal basidiospores spores (Desjardin 1991).

Tetrapyrgos longicystidiata A.H. Honan, Desjardin & T.J. Baroni, Phytotaxa 231: 108 (2015)

FIG. 6

Pileus 10–15 mm diam, plane, broadly convex to convex; greyish-brown to olivaceus (706B53) turning whitish (DBD8C8) to margin, brownish-cream (E0D9B6) when dried; surface sulcate, pruinose; margin incurved, campanulate when young, plane to uplift, dry, opaque. *Lamellae* adnate, slightly intervenose *L*= 15–18, *l*= 1–3; white-greyish (D1CFC2). *Stipe* 10–18 × 0.5–1.5 mm; central, cylindrical, thin, thicker at apex, circular to depressed,

horny, flexible, hollow; black turning white to apex with granular white covering; narrow mycelia pad, greyish-olivaceus (545248). Odor and taste not observed.

Basidiospores 8−11 × 7−10 µm [X_m= 9.7 ±0.7 × 8.5 ±0.7 µm; *Q*= 1- 1.29, Q_m= 1.3 ±0.1; n=20] tetrahedral, smooth, thin-walled, hyaline, inamyloid. *Basidia* 35−45 × 8−10 µm, clavate, 4-sterigmate. *Basidioles* 32−37 × 6−7 µm, clavate to fusiform. *Pleurocystidia* absent. *Cheilocystidia* main body 25−55 × 4−6 µm; cells elongate, branched, diverticulate, irregular, hyaline, thin-walled; diverticula, 1−11 × 0.5−4 µm, elongate, bifurcated, knob-like, bulboid. *Lamellar trama* interwoven, hyphae 4−5 µm diam, hyaline. *Pileocystidia* main body 30−45 × 3−4 µm, overall diverticulate, rod-like, most cells irregularly branched usually with a bulbous apex 6−12 × 5−10 µm, hyaline, thin-walled; diverticula 1−6 × 0.5−5 µm. *Pileus* trama with interwoven cells, hyaline, 3−5 µm diam. *Stipitipellis* trama with parallel, cylindrical, brownish-golden, thick-walled 3−5 µm diam; some cells 2−3 µm diam, setaceous, bulbous, brownish, thick-walled. *Caulocystidia* 50−100 × 4−5 µm, somewhat similar to pileocystidia but longer, diverticulate, most with bulbous apex, hyaline cells. *Clamp connections* present in all tissues.

Habit and Habitat: Marasmioid, gregarious on undetermined dicotyledonous plants branches at leaf litter in Amazonian terra firme forest.

Distribution: Argentina, Bolivia, British Virgin Island, Costa Rica, Puerto Rico (Honan 2007) and Brazil.

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF−2), 11 May 2012, *D.L, Komura & T.S. Marinho DLK561* (INPA259597); 23 Apr 2013, *D.L. Komura & P.A. Pereira DLK1223* (INPA259596); 24 Apr 2013, *D.L. Komura & P.A. Pereira DLK1250* (INPA259598); *D.L. Komura & O.F. Menezes DLK1534* (INPA259611).

Comments: Our collections are similar to the description of *Tetrapyrgos longicystidiata* in Honan et al. (2015) with longer cheilocystidia (generally >50 µm), and pileocystidia lobes are elongate rather than bulbous at the apex. *T. longicystidiata* also resembles *T. nigripes* (Fr.) E.

Horak by having a whitish and pruinose pileus and diverticulate and bulbous cheilocystidia. ITS data analysis also support our specimens as *T. longicystidiata*, placed both our and Honan (2007) sequences on the same clade.

Tetrapyrgos pileobrunnea D.L. Komura & J-M. Moncalvo sp. nov. FIG. 7

MycoBank MBXXXX

Typification: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF−2), 24 Apr 2013, *D.L.Komura & P.A. Pereira DLK1251* (**holotype** INPA259608). GenBank accession : ITS KT287097.

Etymology: Based on the brown color of the pileus.

Diagnosis: This species is distinguished from others of the genus by its brownish, opaque, and fuzzy pileus; cream and anastomosed lamellae; and a large greyish disc. The cheilocystidia have cells with thick-walled base and lobate apex.

Description: Pileus 10−23 mm diam, convex to broadly convex, striated; dark-brown at center (301B05) and brown lines; fuzzy, opaque; in the herbarium turning rust- brown (8A460F). *Lamellae* adnate, intervenose, some forked; cream (E0D9B6), *L*= 16−18; three series of lamelullae, *l*= 4−5. *Stipe* 11−21× 0.5−1.5 mm, central, thicker at apex, circular to depressed, horny, flexible; black, turning cream to upper, covered by white, bright granules; attached to a large mycelial disc grey (828075), turning pale when dried (FADEA2).

Basidiospores 7−15 × 5−12 µm [X_m= 9.8 ±2.5 × 7.7 ±2.2 µm; *Q*=1.2−1.8, Q_m= 1.3 ±0.2; n=15], tetrahedral, some views are triangular, smooth, thin-walls, hyaline, inamyloid. *Basidia*, 30−32 × 8−10 µm, clavate, 4-sterigmate. *Basidioles*, 30−35 × 5−7 µm, clavate, some fusiform. *Pleurocystidia* absent. *Cheilocystidia* main body, 24–48 × 10−17 µm; some clavate with rod-like apex, most cells diverticulate, other with large, thick-walled base, with diverticulate cells on the middle, and apex lobate to bulbous, versiform, hyaline, thin-walled,

diverticula 0.5−1 × 0.5−5 µm. *Lamellar trama* interwoven, cells 3–4.5 µm diam, cylindrical, smooth, hyaline. *Pileocystidia* main-body 40−70 × 5–7 µm, overall lobate, rod-like, most cells irregular, usually with a bulbous apex, hyaline, thin-walled, some clavate, thick-walled; diverticula 6−2 × 0.5−6 µm, obtuse, some bifurcate, lobate. *Pileus trama* interwoven cells, hyaline, 5.2–6.9 µm diam thick-walled, brownish cells. *Stipitipellis* parallel, cylindrical, thick-walled, brownish, some setaceous; cells 2–3 µm diam, thick-walled, brownish. *Caulocystidia* 40–70 × 7–10 µm, diverticulate, bulbous, some branched, clavate apex, hyaline cells, thin-walled; diverticula, 1–10 × 0.5–3 µm, obtuse, pyriform, bulbous, some branched. *Clamp connections* not observed.

Habit and Habitat: Marasmioid, gregarious on branches of undetermined dicotyledonous plants on the leaf litter in Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Additional Specimens Examined: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF–2), 24 Apr 2013, *D.L.Komura & P.A. Pereira DLK1255* (INPA259609); *DLK1306* (INPA259610).

Comments: This species is distinguished from the other known *Tetrapyrgos* species by having a brownish, opaque, fuzzy pileus, cream and anastomosed lamellae, and a large greyish disc where the stipe is attached the substrate. The cheilocystidia are thick-walled at the base and have lobes at the apex. Singer (1973) reported a form of *Marasmiellus nigripes* (Fr.) Singer (syn. *T. nigripes*) from Mexico with a pileus dark grey blackish at the center, spores tetrahedral, cruciform and with elongate, diverticulate, clavate cheilocystidia that could resemble our species.

Tetrapyrgos pseudonigripes D.L. Komura & J-M. Moncalvo sp. nov. FIG. 8

MycoBank MBXXXX

Typification: BRAZIL, AMAZONAS, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 10 May 2012, *D.L.Komura & T. Marinho DLK459* (**holotype** INPA259599). GenBank accession: ITS KT287084.

Etymology: The specimen resembles the *T. nigripes* described to USA, North Carolina (Type locality).

Diagnosis: This species is distinguished by a reduced number of cheilocystidia, and lack of diverticulas.

Description: *Pileus* 3 mm diam, convex, pruinose, white-greyish, slightly (E1F2E6) olivaceus, opaque; brownish (826534) in dried material. *Lamellae* adnate, few, *L*= 8, *l*= 1 serie, white; *Stipe* 10 × 0.5 mm, central, cylindrical, thin, circular, horny, flexible, hollow; entirely black with coiled white covering.

Basidiospores 10–13 × 7–11 µm [X_m= 10.8 ±1.1 × 9.4 ±1.3 µm; *Q*= 0.9–1.4, Q_m= 1.2 ±0.1; n=13], tetrahedral, smooth, thin-walls, hyaline, inamyloid. *Basidia*, 30–32 × 8–10 µm, 4–sterigmate, clavate. *Basidioles*, 28–37 × 7–10 µm, clavate, some elongate. *Pleurocystidia* absent. *Cheilocystidia*, few, main body 16–64 × 7–14 µm, branched, not diverticulate, versiform, hyaline, thin-walled, bifurcated, lobate. *Lamellar trama* regular, interwoven cells 4–5 µm diam, hyaline. *Pileocystidia* main body 25–45 × 4–17 µm, diverticulate overall, thick-walled, others elongate, lobate, diverticulate, irregular, thin-walled, hyaline. *Pileus trama* irregular with interwoven cells, hyaline, 4–5 µm diam. *Stipitipellis* trama with parallel hyphae, cylindrical, brownish golden, thick-walled 4–10 µm diam. *Caulocystidia* main body 20–35 × 5–10 µm, diverticulate, lobate, hyaline cells; diverticula 0.5–5 × 0.5–2 µm, obtuse, bifurcate, hyaline; lobes 5–10 × 3–5 µm, hyaline, thin-walled, some thick-walled, brownish, arising direct from the parallel stipitipellis trama.

Clamp connections present in all tissues.

Habit and Habitat: Marasmioid, solitary on branches of dicotyledonous plants at leaf litter in Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Additional Specimens Examined: BRAZIL, AMAZONAS, Barcelos, Rio Aracá, 29 Jan 2015, *J.J.S Oliveira; J.R.Barbosa; D.L.Komura AM15064* (INPA265162); Manaus, 15 Feb 2015 *O.S.Pereira; J.J.S.Oliveira; A.K.N.C.Silva & D.L.Komura AM15444* (INPA265320).

Comments:

Our material is very similar to that of *T. albonigripes*, but it has a smaller pileus, up to 3 mm; distant lamellae, up to 8; and lobate cheilocystidia. *T. pseudonigripes* is also similar to *T. nigripes*, as they share lamellae disposition (9- 13), a central stipe and a pruinose, white pileus. The three species cited above have, in common, a pileipellis compose by Rameales-like structure.

KEY TO THE KNOW AMAZONIAN SPECIES OF *TETRAPYRGOS*

* Pileus white, brown without any olivaceus or greyish tint……..…**I**

* Pileus white, cream, greyish, olivaceus or greyish tint present…….....……...**II**

I.1a Pileus browm, fuzzy, dull, opaque…… ***T. pileobrunnea***

I.1b Pileus brown, cream or white, smooth, shinny……..…… **I. 2**

I.2a Pileocystidia irregularly branched and elongate…… ***T. brunneilucida***

I.2b Pileocystidia shorter than other species in the genus, up to 30 μm long…..... ***T. brevipileocystidiata***

II. 1a Pileus greyish brown to olivaceus pileocystidia with some cells thick-walled or with thin excrescences covering theses cells …….....…***T. cystidiacrassa***

II. 1b Pileus prevailing white greyish or cream and pileocystidia not thick-walled neither with excrecences… ……..……..……II. 2

II. 2a Pileus with greyish to brownish disc, not turning cream when dry and cheilocystidia predominantly longer than other species in the genus, > 50 μm long…….…. ***T. longicystidiata***

II.2b Pileus with no greyish to brownish disc and cheilocystidia predominantly < 50 μm long……..**II.3**

II.3a Pileus when dry keep pale to cream color, cheilocystidia, abundant, diverticulate and lobate apex……..….***T. albonigripes***

II.3b Pileus when dry turning brown, cheilocystidia, few, lack of small diverticula……

……..…***T. pseudonigripes***

DISCUSSION

We described six new *Tetrapyrgos* species from the Brazilian Amazon and provided an identification key for the seven species currently known from the Amazon basin. The distinction between these species is supported from both morphology and ITS-rDNA barcodes (Schoch et al. 2012).

Although two of our new taxa indeed fit into the broad morphological concept of *T. nigripes* (Singer 1973, Horak 1983), we describe them herein as two distinct species (*T. albonigripes* and *T. pseudonigripes*) based on combined evidence from ITS sequence and morphology. We also retrieved several sequences from Genbank from various geographic origins that were labeled *T. nigripes* or *T.* aff. *nigripes* (Table 1), that did not cluster together in the ITS tree depicted in Fig. 1 but segregate geographically. For now, because *T. nigripes* was described from the U.S.A., we consider the Genbank sequences that originated from this country to correspond to the "true" *T. nigripes*, and hypothesize that the Australian and Hawaiian collections referring to this species represent different, yet unnamed cryptic species.

Tetrapyrgos nigripes (Fr.) E. Horak also was described from Argentina, Venezuela and Papua New Guinea. It is reported to be highly variable both macro- and microscopically, and to have a broad geographic distribution, especially in the subtropical, tropical and temperate zone of the South America (Singer 1973, Horak 1983). Singer (1973) informally recognized five forms based on pileus color variations from white to dark grey with a blackish center, and whether or not it turns blue or green when bruised, including some forms with a pleurotoid habit. Horak (1983) examined microscopic features of specimens from Argentina and Papua New Guinea and describe many differences in cystidia types, including the presence of pleurocystidia, these structure are not present in our specimens studied.

Additional *Tetrapyrgos* species certainly remain to be properly described and named for our Amazonian collections. For instance, ITS sequences produced from two specimens (AM15316 *T.* sp1 and DLK1970 *T.* sp2) stand alone (Fig. 1), suggesting that they probably represent undescribed species. However, these two collections are not of excellent quality. We therefore refrain to formally describe and propose them as new species. We also retrieved two environmental sequences from Genbank (JN890461, JN890458) that were obtained from a forest soil in Guyana (McGuire et al. 2010) that strongly cluster together on a lone branch within *Tetrapyrgos* (Fig. 1). They almost certainly represent a still undescribed *Tetrapyrgos* species, of which a fruiting body has yet to found and/or ITS-rDNA barcoded.

Overall, this study provide evidence that additional *Tetrapyrgos* species are yet to be discovered, and supports the fact that new fungal species can be first discovered from DNA-based studies of environmental samples rather than from traditional fieldwork (Porter et al. 2008, Fischer et al. 2012), as indicated from the sequences obtained by McGuire et al. (2010) from a Guyana soil (Fig.1). This study also suggests the presence of several cryptic species within *Tetrapyrgos* that were not recognized from standard morphological examination. For instance, the five ITS sequences labeled *T. subdendrophora* retrieved from Genbank show strong phylogenetic structure as they are split into two clades (Fig. 1). Indeed showing sequence variation (08%) higher than the generally observed ITS interspecies variation among fungi (0–3%; Nilsson et al. 2008), suggest the existence of cryptic species within this taxon. Similarly, the fifteen sequences labeled *T. subcinerea* that we retrieved from Genbank strongly split into two groups that differ by 1–4%.

The morphological distinction between *Tetrapyrgos* and *Campanella* still remains unclear (see Honan 2007, Honan et all. 2015 for a discussion), as also supported from ITS phylogeny. For example, ITS sequences indicate that *T. subdendrophora* is intermediate between the *Tetrapyrgos* and *Campanella* clades (Fig. 1). In addition, in the course of this study, we retrieved ITS sequences from Genbank labeled *Marasmiellus candidus* (EF175516,

EF175514, EF175513), *Marasmiellus paspali* (EF175515, EF175512, EF175511), *Marasmius tricolor* (KJ188733, JN943601), and several "environmental sequences" that cluster within the *Tetrapyrgos*/ *Campanella* clade.

Clearly, ITS sequences alone are insufficient for generic segregation and taxonomy in this clade, especially until the molecular data from the respective types of *Tetrapyrgos*, *Campanella* and *Marasmiellus* will be produced. In addition, available high quality collections are necessary to link the relationship of the morphological hypotheses with molecular evidence.

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FIGURE LEGENDS

FIG. 1. Maximum likelihood tree and Bayesian analysis based on ITS (ITS1-5.8S-ITS2) dataset sequences of *Tetrapyrgos* spp. The Bayesian posterior probabilities value–PP and maximum likelihood bootstrap percentage–BS are along branches, with value separated by "/", respectively. Branches in bold green received high support in both analysis; branches in bold blue received moderate to high support in at least one of the analysis. Scale bar value indicates nucleotide substitutions/site. Newly sequenced collections are labeled with initial DLK or AM. *Marasmius rotula* was used as outgroup.

FIG. 2. *Tetrapyrgos albonigripes*. A–A3. Basidiome from DLK1360– Holotype. B–B1. Basidiome (DLK1699). C. Basidiospores. D. Basidium and Basidiole. E. Cheilocystidia. F. Pileocystidia. G. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.3. *Tetrapyrgos brevipileocystidiata.* A–A1. Basidiome from DLK1602– Holotype. B–B1. Basidiome (DLK1065). C. Basidiospores. D. Basidia and Basidiole. E. Cheilocystidia. F. Pileocystidia. G. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.4. *Tetrapyrgos brunneilucida*. A–A2. Basidiomes from DLK1339– Holotype. B–B1. Basidiome (DLK1213). C. Basidiospores. D. Basidioles and Basidia. E. Cheilocystidia. F. Pileocystidia. G. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.5. *Tetrapyrgos cystidiacrassa* (DLK336– Holotype). A–A4. Basidiome. B. Basidiospores. C. Basidioles. D. Cheilocystidia. E. Pileocystidia. F. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.6. *Tetrapyrgos longicystidiata* (DLK1250). A–A2. Basidiomes. B. Basidiospores. C. Basidia and Basidioles. D. Cheilocystidia. E. Pileocystidia. F. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.7. *Tetrapyrgos pileobrunnea* (DLK1251– Holotype). A–A3. Basidiomes. B. Basidiospores. C. Basidioles and Basidia. D. Cheilocystidia. E. Pileocystidia. F. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FIG.8. *Tetrapyrgos pseudonigripes* (DLK459– Holotype). A–A1. Basidiome. B. Basidiospores. C. Basidia and Basidioles. D. Pileocystidia. E. Cheilocystidia. F. Caulocystidia. Scale bar: 10 μm. Illustrated by D.L.Komura.

FOOTNOTES

[1] Corresponding author. E-mail: leimybio@gmail.com

TABLE I. Strains and GenBank accessions of ITS sequences used in this study.

Species	collection ID	Herbarium accession No.	Location	GenBank Accession No.	Reference
C. olivaceonigra (E. Horak) T.W. May & A.E. Wood		AQ793973	Australia	JX444167	Lebel, McMullan-Fischer 2014; unpublished
Campanella alba (Berk. & M.A. Curtis) Singer		TFB12565	USA,Tennessee	DQ449943	Lickey, Hughes, Petersen 2006; unpublished
Campanella pustulata (Berk. & Broome) Lloyd		AQ793972	Australia	JX444168	Lebel, McMullan-Fischer 2014; unpublished
Campanella sp.	AHH42		Malaysia	EF175519	Honan, Desjardin 2007
Campanella sp.	MCA2235		Guyana	AY916676	Aime, Phillips-Mora 2005
Campanella sp1	DLK1716	INPA270736	Brazil, Amazon	not deposited	this work
Marasmiellus cubensis	DLK1719	INPA259718	Brazil, Amazon	not deposited	this work
Marasmiellus cubensis	DLK1050	INPA271943	Brazil, Amazon	not deposited	this work
Marasmiellus volvatus	DLK1502	INPA259716	Brazil, Amazon	not deposited	this work
Marasmiellus volvatus	DLK417	INPA271932	Brazil, Amazon	not deposited	this work
Marasmiellus candidus	AHH157		USA-California	EF175513	
Marasmiellus candidus			French	EF175516	
T. albonigripes	DLK1360	INPA259599	Brazil, Amazon	KT287081	this work
T. albonigripes	DLK1699	INPA259605	Brazil, Amazon	KT287082	this work
T. albonigripes	DLK1453	INPA259603	Brazil, Amazon	KT287083	this work

T. pseudonigripes	DLK459	INPA259600	Brazil, Amazon	KT287084	this work
T. pseudonigripes	AM15064	INPA265162	Brazil, Amazon	KT287085	this work
T. pseudonigripes	AM15444	INPA265320	Brazil, Amazon	KT287086	this work
T. aff. *nigripes*	GMB-2014	MEL:2382866	Australia	KP012740	Bonito et al. 2014; unpublished
T. aff. *nigripes*	GMB-2014	MEL:2382974	Australia	KP012833	Bonito et al. 2014; unpublished
T. brevipileocystidiata	DLK1065	INPA 270737	Brazil, Amazon	KT287087	this work
T. brevipileocystidiata	DLK1602	INPA259604	Brazil, Amazon	KT287088	this work
T. brunneolucida	DLK1213	INPA259601	Brazil, Amazon	KT287089	this work
T. brunneolucida	DLK1339	INPA259594	Brazil, Amazon	KT287090	this work
T. cystidiacrassa	DLK374	INPA259607	Brazil, Amazon	KT287091	this work
T. cystidiacrassa	DLK336	INPA259606	Brazil, Amazon	KT287092	this work
T. longicystidiata A.H. Honan, Desjardin & T.J. Baroni	DLK1223	INPA259596	Brazil, Amazon	KT287093	this work
T. longicystidiata	DLK1250	INPA259598	Brazil, Amazon	KT287094	this work
T. longicystidiata	DLK1534	INPA259611	Brazil, Amazon	KT287095	this work
T. longicystidiata	DLK561	INPA259597	Brazil, Amazon	KT287096	this work
T. longicystidiata	TJB7902			EF175542	Honan, Desjardin 2007
T. longicystidiata		NYBG6376	Bolívia	EF175533	Honan, Desjardin 2007
T. longicystidiata	ZT12385		Costa Rica	EF175543	Honan, Desjardin 2007

T. longicystidiata	TJB7935		Puerto rico	EF175544	Honan, Desjardin 2007
T. longicystidiata		NYBG8396	Costa Rica	EF175545	Honan, Desjardin 2007
T. nigripes (Fr.) E. Horak		TFB12137	USA,Tennessee	DQ449941	Lickey, Hughes, Petersen 2006; unpublished
T. nigripes		TFB12583	USA,Tennessee	DQ449942	Lickey, Hughes, Petersen 2006; unpublished
T. nigripes	Wong888		Hawaii	EF175535	Honan, Desjardin 2007
T. nigripes	TOR89		British Virgin Islands	EF175540	Honan, Desjardin 2007
T. parvispora A.H. Honan, Desjardin & T.J. Baroni	AHH66		Thailand	EF175536	Honan, Desjardin 2007
T. parvispora	AHH130		Thailand	EF175538	Honan, Desjardin 2007
*T. parvispora**	AHH122		Thailand	EF175551	Honan, Desjardin 2007
T. parvispora	AHH88		Thailand	EF175546	Honan, Desjardin 2007
T. pileobrunnea	DLK1251	INPA259608	Brazil, Amazon	KT287097	this work
T. pileobrunnea	DLK1306	INPA259610	Brazil, Amazon	KT287098	this work
T. pileobrunnea	DLK1255	INPA259609	Brazil, Amazon	KT287099	this work
T. subcinerea (Berk. & Broome) E. Horak	AHH84		Thailand	EF175524	Honan, Desjardin 2007
T. subcinerea	AR505		Indonesia	EF175530	Honan, Desjardin 2007
T. subcinerea	AHH71		Thailand	EF175534	Honan, Desjardin 2007
T. subcinerea	AHH86		Malaysia	EF175537	Honan, Desjardin 2007
T. subcinerea	AR019		Indonesia	EF175548	Honan, Desjardin 2007

T. subcinerea	KUM60047		Malaysia	EF175549	Honan, Desjardin 2007
T. subcinerea	AHH115		Indonesia	EF175550	Honan, Desjardin 2007
T. subcinerea	AHH90		Malaysia	EF175552	Honan, Desjardin 2007
T. subcinerea	AR138		Indonesia	EF175554	Honan, Desjardin 2007
T. subcinerea	DED7448		Thailand	EF175553	Honan, Desjardin 2007
T. subcinerea	KUM60051		Malaysia	EF175527	Honan, Desjardin 2007
T. subcinerea	DED6178		USA	EF175528	Honan, Desjardin 2007
T. subcinerea	AHH129		Thailand	EF175526	Honan, Desjardin 2007
T. subcinerea	DED7517		Malaysia	EF175532	Honan, Desjardin 2007
T. subcinerea	RW832		Papua New Guinea	EF175539	Honan, Desjardin 2007
T. subdendrophora [a]		ATCC42449	Australia	AY445121	Vinnere et al. 2005
T. subdendrophora (Redhead) E. Horak	DED7338		California	EF175529	Honan, Desjardin 2007
T. subdendrophora	AHH120		California	EF175521	Honan, Desjardin 2007
T. subdendrophora	AHH148		California	EF175522	Honan, Desjardin 2007
T. subdendrophora	AHH79		California	EF175523	Honan, Desjardin 2007
Tetrapyrgos sp.1	AM15316	INPA265272	Brazil, Amazon	KT287100	this work
Tetrapyrgos sp.2	DLK1970	INPA270738	Brazil, Amazon	KT287101	this work
Uncultured fungus clone	PEG11GadID		Guyana	JN890461	McGuire et al. 2010
Uncultured fungus clone	PEG07GadID		Guyana	JN890458	McGuire et al. 2010

Marasmius rotula	*out group	Denmark	JN943598	Schoch et al. 2012
Marasmius rotula	*out group	Germany	JN714927	Grobe et al. 2011

[a] labeled as *Campanella subdendrophora* in Vinnere et al. 2005.

*not used in final tree

FIG.1

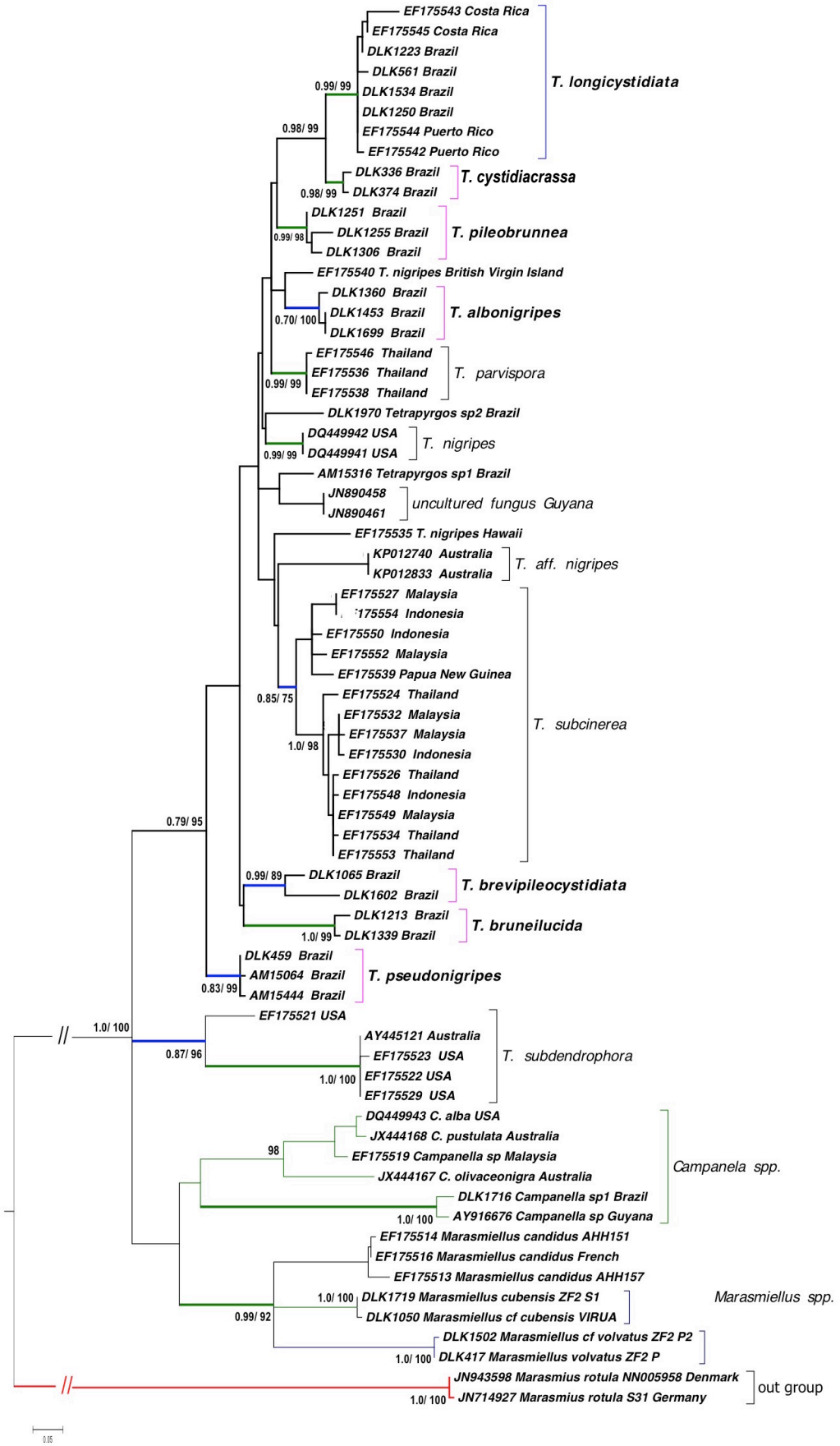

EF175543 Costa Rica
EF175545 Costa Rica
DLK1223 Brazil
DLK561 Brazil
DLK1534 Brazil
DLK1250 Brazil
EF175544 Puerto Rico
EF175542 Puerto Rico
T. longicystidiata
0.99/ 99
0.98/ 99
DLK336 Brazil
DLK374 Brazil
T. cystidiacrassa
0.98/ 99
DLK1251 Brazil
DLK1255 Brazil
DLK1306 Brazil
T. pileobrunnea
0.99/ 98
EF175540 T. nigripes British Virgin Island
DLK1360 Brazil
DLK1453 Brazil
DLK1699 Brazil
T. albonigripes
0.70/ 100
EF175546 Thailand
EF175536 Thailand
EF175538 Thailand
T. parvispora
0.99/ 99
DLK1970 Tetrapyrgos sp2 Brazil
DQ449942 USA
DQ449941 USA
T. nigripes
0.99/ 99
AM15316 Tetrapyrgos sp1 Brazil
JN890458
JN890461
uncultured fungus Guyana
EF175535 T. nigripes Hawaii
KP012740 Australia
KP012833 Australia
T. aff. nigripes
EF175527 Malaysia
175554 Indonesia
EF175550 Indonesia
EF175552 Malaysia
EF175539 Papua New Guinea
EF175524 Thailand
EF175532 Malaysia
EF175537 Malaysia
EF175530 Indonesia
EF175526 Thailand
EF175548 Indonesia
EF175549 Malaysia
EF175534 Thailand
EF175553 Thailand
T. subcinerea
0.85/ 75
1.0/ 98
0.79/ 95
0.99/ 89
DLK1065 Brazil
DLK1602 Brazil
T. brevipileocystidiata
DLK1213 Brazil
DLK1339 Brazil
T. bruneilucida
1.0/ 99
DLK459 Brazil
AM15064 Brazil
AM15444 Brazil
T. pseudonigripes
0.83/ 99
EF175521 USA
AY445121 Australia
EF175523 USA
EF175522 USA
EF175529 USA
T. subdendrophora
1.0/ 100
0.87/ 96
1.0/ 100
DQ449943 C. alba USA
JX444168 C. pustulata Australia
EF175519 Campanella sp Malaysia
JX444167 C. olivaceonigra Australia
DLK1716 Campanella sp1 Brazil
AY916676 Campanella sp Guyana
Campanela spp.
98
1.0/ 100
EF175514 Marasmiellus candidus AHH151
EF175516 Marasmiellus candidus French
EF175513 Marasmiellus candidus AHH157
DLK1719 Marasmiellus cubensis ZF2 S1
DLK1050 Marasmiellus cf cubensis VIRUA
Marasmiellus spp.
1.0/ 100
0.99/ 92
DLK1502 Marasmiellus cf volvatus ZF2 P2
DLK417 Marasmiellus volvatus ZF2 P
1.0/ 100
JN943598 Marasmius rotula NN005958 Denmark
JN714927 Marasmius rotula S31 Germany
out group
1.0/ 100
0.05

FIG.2 *Tetrapyrgos albonigripes*

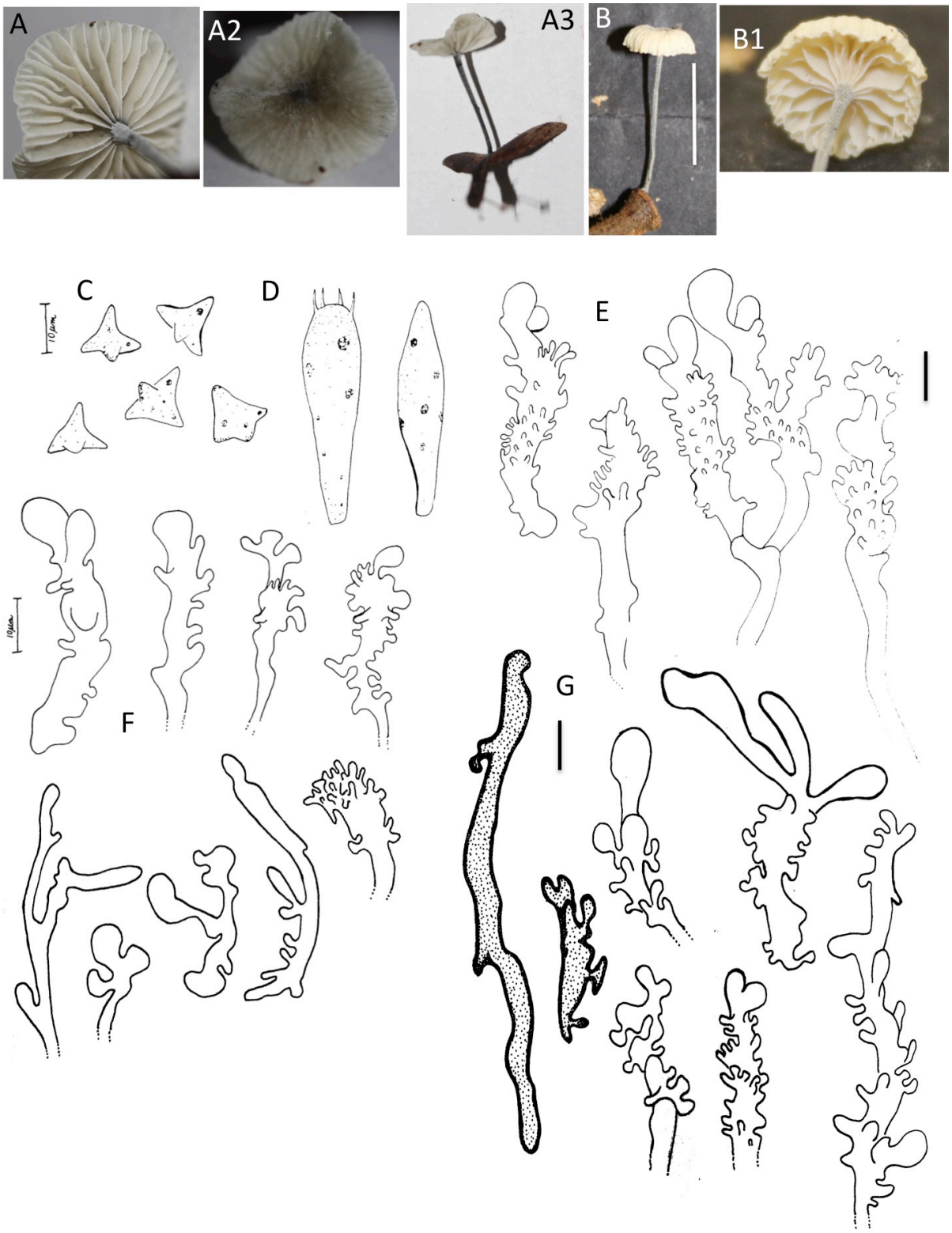


FIG.3 *Tetrapyrgos brevipileocystidiata*

FIG.4 *Tetrapyrgos brunneilucida*

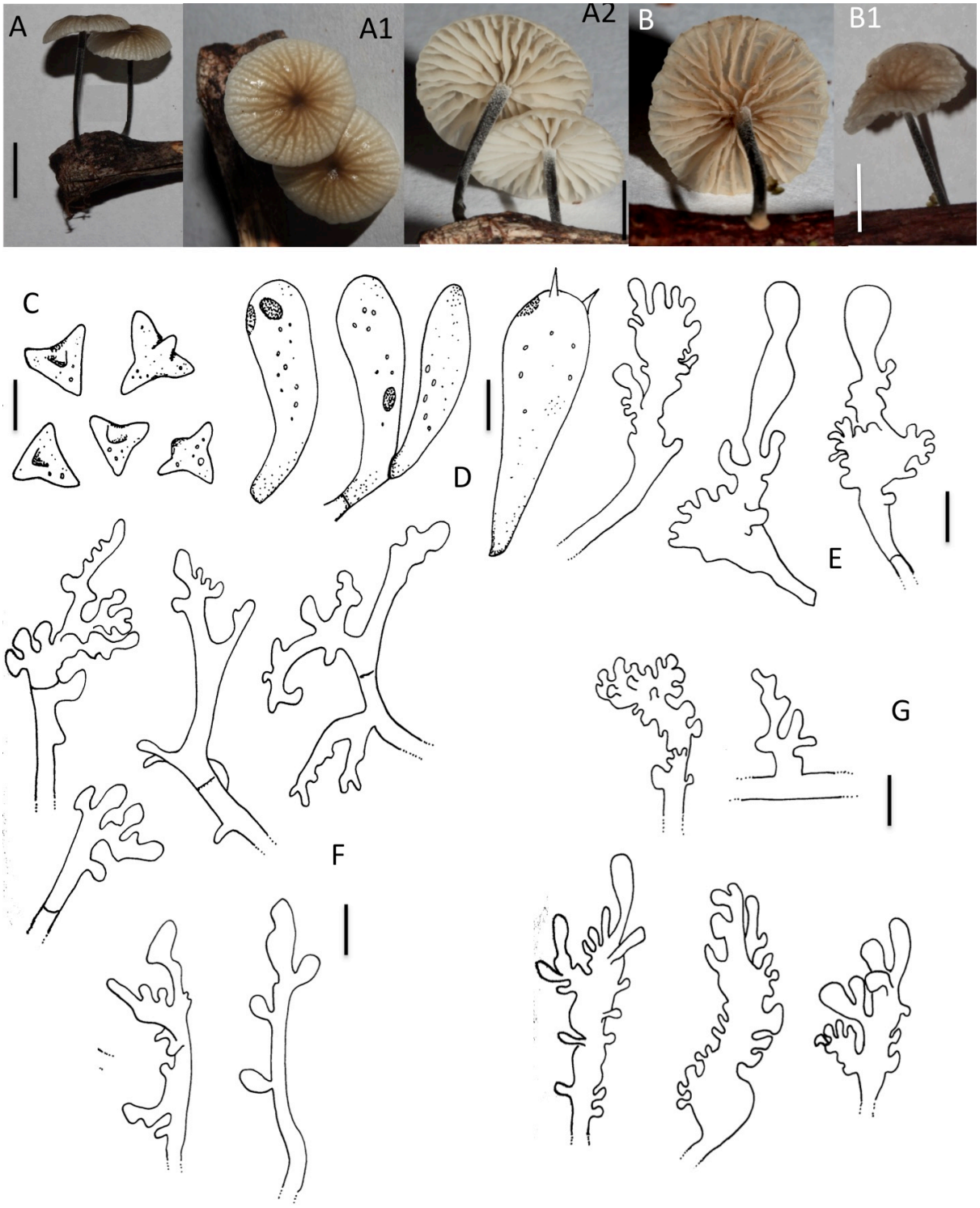


FIG.5 *Tetrapyrgos cystidiacrassa*

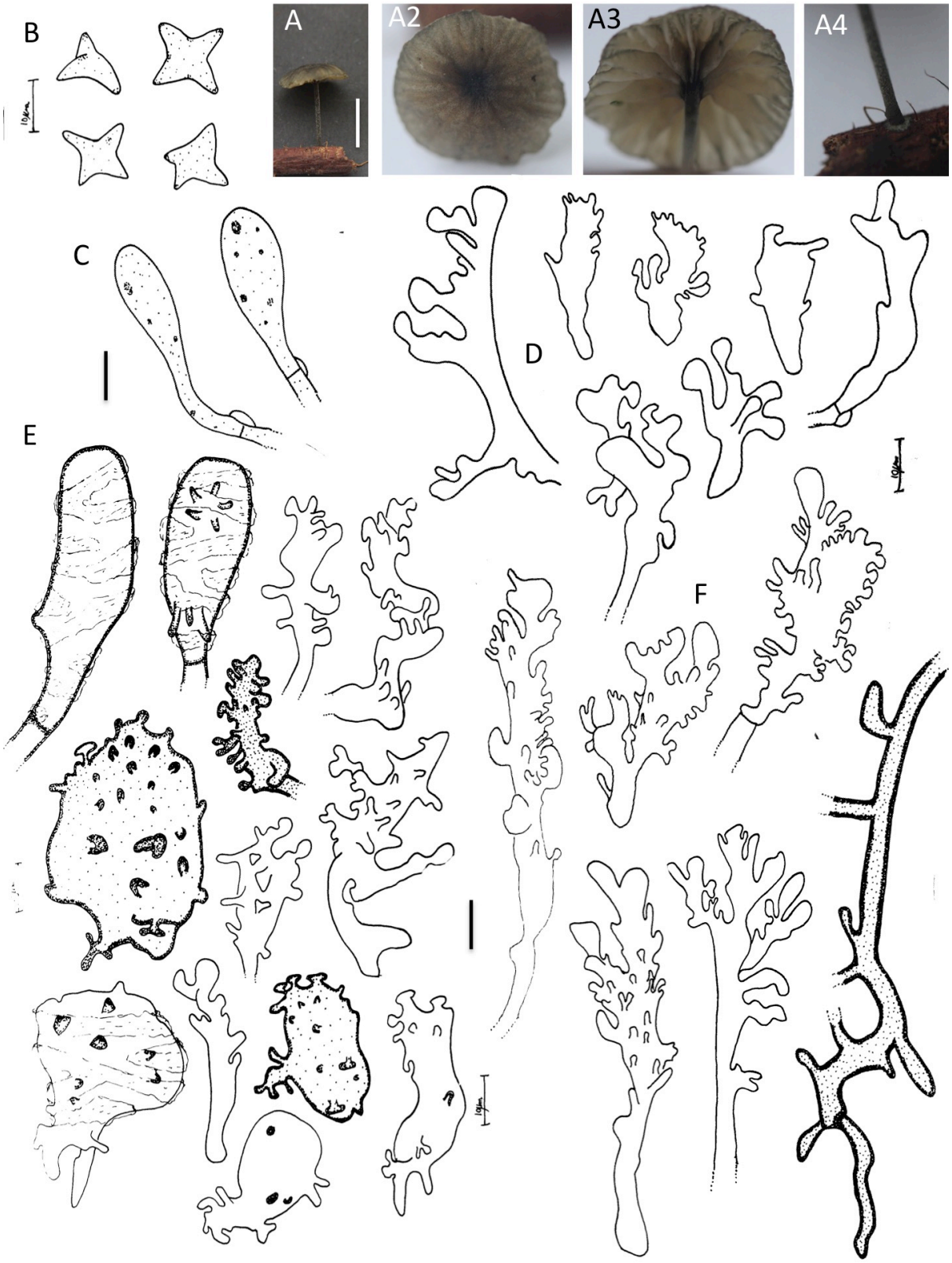
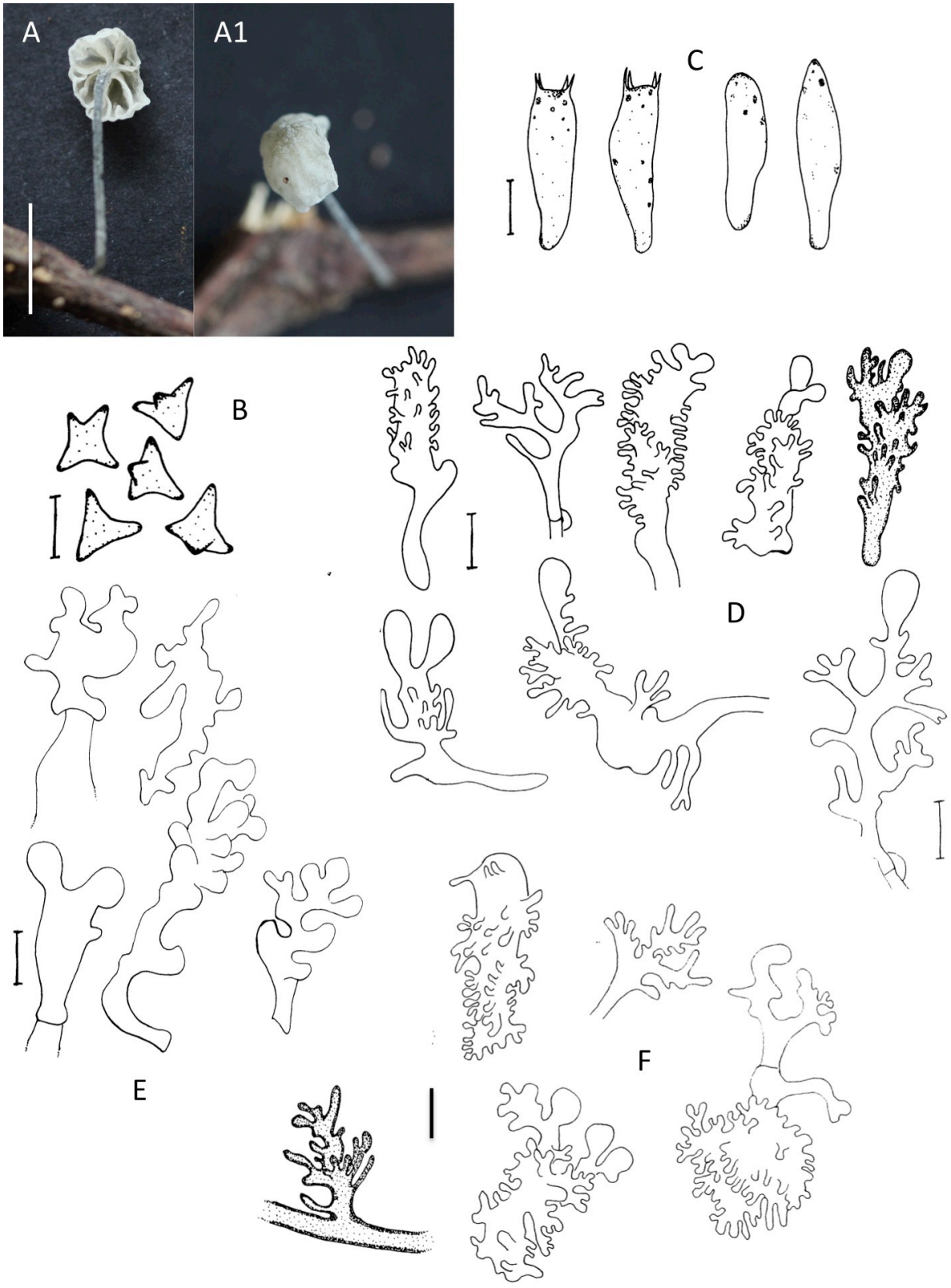


FIG.6 *Tetrapyrgos longicystidiata*

Fig. 7 *Tetrapyrgos pileobrunnea*

FIG. 8 *Tetrapyrgos pseudonigripes*



Capítulo 3

Komura, D.L; Oliveira, J. J. S. Moncalvo, J. M.; Margaritescu, S.; Zartman, C. E. *Marasmius calvocystidiatus* sp. nov. and *M. horridulus* from Amazon forest: two unusual species of sect. *Marasmius*

Manuscrito em preparação para *Phytotaxa*

Marasmius calvocystidiatus sp. nov. and *M. horridulus* from Amazon forest: two unusual species of sect. *Marasmius*

DIRCE L. KOMURA[1*]; JADSON J. S. OLIVEIRA[2]; JEAN-MARC MONCALVO[2]
SIMONA MARGARITESCU[2] & CHARLES E. ZARTMAN[1]

[1]*Instituto Nacional de Pesquisas da Amazônia (INPA), Botany Dept., C.P. 478, 69011-970 Manaus, Amazonas, Brazil.*

[2]*Depart. of Natural History, Royal Ontario Museum, and Dept. of Ecology and Evolutionary Biology, University of Toronto, Canada*

** Corresponding author: leimybio@gmail.com*

Abstract

Marasmius calvocystidiatus sp. nov. is proposed as a new species in *Marasmius* sect. *Marasmius*. The smooth cystidia, some lobulated or presenting coarse excrescences composing the pileipellis is a very unusual pattern in the section and diagnostic for the species. Its recognition as belonging to *Marasmius* is confirmed by its phylogenetic assessment using ITS. The new species showed itself more close to members of sect. *Marasmius*, especially as sister of *M. horridulus*, another unusual species in the section, the only one representative of sect. *Horriduli* so far. In this paper, morphological description, taxonomic discussion, line drawing illustration and color plates are provided for both M. *calvocystidiatus* and *M. horridulus*. Either the ITS sequences of both species serve as barcode or to verify their placement among closely related species of the section within a tree.

Key words: Marasmiaceae, taxonomy, ITS, phylogeny, Neotropic, Amazon forest,

Introduction

Marasmius Fr. (Marasmiaceae) contain around 500 species, has a large distribution, especially throughout the tropic (Kirk *et al.* 2008). They are usually tiny mushroom inhabiting the leaf litter on the forest. The subsection *Horriduli* is within section *Marasmius*, and it was created by Singer (1986) to accommodate just one species, *Marasmius horridulus* Sing. This taxon differs from taxon in the other subsection in the presence of numerous pseudoamyloid setiform hairs, especially along at the edge of the pileus, and it was described to Brazilian Amazon.

The presence of setiform hairs usually leads to misidentification of this species as *Crinipellis* Pat., and this may lead to just the presence of the holotype collection from year 1978 in the Herbarium collection. However *Marasmius horridulus* have characteristics that resemble to *Marasmius* species, based to the Singer protologue, this species have collariate lamellae; insititious, glabrous and filiform stipe; pilear surface hymeniform to subhymenform, with short cells, thick-walled and apical, thick walled setullae that somewhat resemble to *Siccus* type broom cells, between very long hairs (Singer 1989).

And now, based on morphological and ITS sequences we report a second collection of *M. horridulus* and describe a new species close related, *M. calvocystidiatus*, providing pictures, illustration of those taxa.

Material and methods

Areas sampled

The specimens were collected at three sites in Brazil: 1) Estação Experimental de Manejo Florestal do INPA (ZF-2), Manaus, Amazonas State (02°37’ and 02°38’S; 60°09’ and 60°11’W), 2) Reserva de Desenvolvimento Sustentável do Tupé, Manaus State (03° 07' S, 60°

18' W) and 3) Floresta Nacional do Tapajós, ICMBio– Base Terra Rica, km 67, Belterra, Pará State (02°51'37"S and 54°57'57").

Morphological analyses

Macroscopic characteristics were described based on fresh material before dried at around 40 °C and, subsequently, microscopic features were examined. Color data were from Color Picker (http://www.colorpicker.com). The microscopic observation was carried out according to Oliveira and Capelari (2012). Sections of dried material were rehydrated in 70% ethanol and mounted in 5% KOH or in Melzer reagent for the amyloidity test. The dimensions of the spore measurements included the range of length × width, and following statistical analysis: χ_m, the arithmetic mean of length × width ± standard deviation; Q_m, the mean of the range of length/width of basidiospores ± standard deviation; and n, the number of spores measured. The lamellae spacing was determined by the following factors: L, the number of lamellae that reach from the stipe to the pileus margin; and l, the number of series of lamellulae among the lamellae. The dried collections were deposited in the INPA and duplicates in the ROM herbaria.

Sequencing and phylogenetic analyses

DNA extraction and the PCR and sequencing of the ITS (ITS1-5.8S-ITS2) followed the method described in Dentinger *et al.* (2010) at Laboratory of Molecular Sistematic from Royal Ontario Museum, Toronto, CA. The sequencing was carried out in an ABI3730 automatic DNA sequencer (Applied Bio-systems). Sequences were assembled/edited using Sequencher v. 4.1.4 (Gene Codes Corp.). Searches were conducted in the NCBI database using BLAST (Altschul *et al.* 1990). Retrieving sequences with over 90 % of similarity (e-value equal or more closed to 0) compared with the newly produced ITS sequences. Only identified sequences assigned to members of sect. *Marasmius* passing by quality and identity

filtering (based on the alignment and preliminary analyses) and preferably published in studies presenting reliable taxonomic counterpart (Wannathes *et al.* 2009, Antonín *et al.* 2014) were retained for the ultimate analyses. The final dataset was arranged to span all infrasectional groups within the section according to the traditional view. The alignment was done using MUSCLE (Edgar 2004) online through EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/muscle/). The preliminary analyses were conducted in MEGA version 6 (Tamura *et al.* 2013) using Maximum-Likelihood (ML) and default parameters. From initial dataset, 37 ITS sequences (Table I) were deemed to be the ingroup, including members of *Crinipellis* and *Chaetochalathus* to compose the outgroup (Wannathes *et al.* 2009, Tan *et al.* 2009). The nucleotide substitution model was selected using MrModeltest 2.3. (Nylander 2004), based on Akaike Information Criterion (AIC).

For Maximum Likelihood (ML), GTR+Γ+I model plus fast-bootstrapping implementing CAT approximations for 1,000 pseudoreplicates and a full ML optimization for the final tree were implemented in RaxML 7.0.4 (Stamatakis 2006). We performed MC^3 Bayesian analysis (BA) with MrBayes 3.2.1 (Ronquist *et al.* 2012). Implementing the GTR+G+I model setting 6 for substitution number, the BA consisted of two independent runs of 5,000,000 generations, sampling frequency every 500 generations, 6 independent chains and 2 swaps. The burning was set at 10 %. Final trees were summarized using the 50 % majority-rule consensus method. Branch lengths were summarized across the 95 % highest posterior density trees. The tree generated by the BA was chosen to display the phylogenetic relationships among the taxa in the final dataset.

Results

ITS sequences analysis

The final dataset consisted of 46 sequences (Table I) and phylogenetic relationship among the taxa generated by the BA is depicted in final tree (Figure 1). Samples from the type specimen of *M. horridulus* to DNA extraction were not available, but the macro and micromorphology

of the new collection indicate that correspond to this species.

The ITS data could not give enough information about the evolutionary relationship within *Marasmius*, but we can infer that these specimens are closer among *Marasmius* species, than *Crinipellis* and *Chaetochalathus* with 88% of Bootstrap support (BS) in Maximum-Likelihood (ML) and 1.0 posteriori probability (PP) in Bayesian (B) analysis.

ITS sequences from *Marasmius calvocystidiatus* (3 collections) and *Marasmius horridulus* (one collection) ITS when submitted to ML and Bayesian analysis showed clearly that represents two distinct species and placed both species in the same clade with high support, 90% BS and 0.99 PP, respectively for the analysis above. *Marasmius horridulus* is close related to *M. purpureisetosus* (100% ML-BS and 1.0 B-PP).

Taxonomy

Marasmius calvocystidiatus D.L.Komura & J.S.Oliveira sp. nov.

[Mycobank XXXX]

Figs. 2, 4 and 5

Diagnosis:— Pileus plane to slightly broadly convex, sulcate, center slightly depressed with a conspicuous papilla, chestnut brown to pale brown, glabrous, dry, opaque, rigid aspect. Lamellae collariate, with very developed collar, lamellae abundant, *L*=32–34, thick and pruinosus, although for the young basidiomata the himenium color is close to pale golden. Stipe central, cylindrical, thin, thinner at apex, circular to depressed, insititious; smooth, glabrous, bright; horny, flexible, hollow; apex whitish to cream, especially when young, restrict to very apical portion when mature, golden brown to reddish brown. This taxa differ from its close related *M. horridulus* by having pileus without setiforms hairs.

Holotype:—BRAZIL. Amazonas State, Manaus District, Estação Experimental de Manejo Florestal do INPA (ZF-2), 23 May 2013, *O.F. Menezes & D.L.Komura DLK1516* (INPA259372).

Etymology:—The pileus surface are smooth, without hair-like structures, and microscopically cystidia are clavate and lobate without setiform structures.

Pileus 2–4 mm diam., plane to slightly broadly convex, sulcate, center slightly depressed with a conspicuous papilla, chestnut brown to pale brown (914809 to F2B068), margin incurved to plane, edge often irregularly ornamented, finely appendiculate, glabrous, dry, opaque, rigid aspect, *Lamellae* collariate, with very developed collar darker (B88651) than lamellae edge (DBAB76), which are next and abundant, *L*=32–34, thick and pruinosus, although for the young basidiomata the himenium color is next to pale golden (FADD7F), lamellae face white to cream (F7E6AD), lamellae on the pileus edge are slightly gelatinous and lighter than lamellae (edge color F7EFB7). *Stipe* 10–14 × 0.2–0.3 mm, central, cylindrical thin, thinner at apex, circular to depressed, insititious; smooth, glabrous, bright; horny, flexible, hollow; apex whitish to cream, especially when young, restrict to very apical portion when mature, golden brown (9E6815) to reddish brown (963A08) elsewhere, base generally darker, bronze (B8860B) when dried.

Basidiospores 4.2–5.2 × 2.6–3.9 µm [X_m= 4.6±0.3 × 3.2±0.4 µm; Q_m= 1.5±0.1; n=15], elliptic to obovoid, smooth, thin-walled, hyaline, inamyloid. *Basidia* clavate, 4–sterigmate. *Basidioles* claviform to filiform, around 20 × 5 µm. *Pleurocystidia* absent. *Cheilocystidia* somewhat similar to *Siccus*-type broom cells, but with more short diverticula, ochraceous, abundant, main body 5–15 × 10–20 µm, most long pedicellate, some clavate, pyriform, thick-walled, cooper brown. *Lamelar trama* dextrinoid, irregular, hyphae thin wall. *Pileus* trama dextrinoid, irregular, includes the edge trama of the pileo, hyphae with clamp connections. *Pileipellis* hymeniform to irregularly hymeniform, cells often smooth all over, many with some apical excrescences, sometimes irregular and lobed, mostly regular when clavate, pyriform, some long pedicellate, deepening in the pileus trama, thick-walled, ochraceous brown to coppery brown, sometimes golden in the cells lumen. *Stipe trama* with parallel, cylindrical, brownish

golden, thick-walled. *Clamp connection* present in all tissues, but the cells of the pileipelis. *Stipitipellis* without differentiated structures.

Additional Specimens Examined:— BRAZIL. Amazonas State, Manaus, Estação Experimental de Manejo Florestal do INPA (ZF-2), 18 April 2012, J.M. Moncalvo, C.E. Zartman, D.L.Komura 307 (INPA270734); Pará State, Belterra, Floresta Nacional de Tapajós, 28 Mar. 2014, *D.L. Komura, T.S. Cabral, I.R. Fonseca DLK1945* (INPA259374).

Habit and Habitat:—marasmioid, gregarious on dicotyledonous leaf on leaf litter at Amazonian terra firme forest.

Distribution:—Brazil, Amazonas and Pará States.

Comments:—This species is easy to recognize in the field, even tiny size, its color brown to golden are maintained even dried, the basidiomes are more horny and rigid than other *Marasmius*, have a conspicuous papilla and thick, close, collariate lamellae. This kind of lamellae is exactly the same found in *Marasmius horridulus*, that differs in the presence of setiformis structure and hair at pileus. *Marasmius calvocystidiatus* also has smooth pileus with pileocystidia clavate, lobate and excrescent pileocystidia, but never acute. The cheilocystia in both species resemble to *Siccus*-type broom cells with brownish golden color.

Marasmius horridulus Singer, The Agaricales in Modern Taxonomy: 367 (1986)

[MB#131519]

Holotype: INPA, Singer & Aguiar (B11272), 1978. **Type locality:** Brazil, Amazonas, 30 km N of Manaus.

Figs.3 and 6

Pileus 3 mm diam., plane to slightly broadly convex, center slightly depressed with a conspicuous papilla, covered densely with long hairs, brown to dark brown (634017), hairs reach beyond the pileous margin. *Lamellae* collariate, with very developed collar dark (4F3211) as well the lamellae edge (4F3211), which are close and abundant, *L*=24, thick and

pruinosus. *Stipe* 28 × 0.3 mm, central, cylindrical thin, circular to depressed, insititious; smooth, glabrous, bright; horny, flexible, hollow, dark brown (4F3211).

Basidiospores not observed. *Basidioles* 20–30 × 5–7 µm. *Pleurocystidia* absent. *Cheilocystidia* somewhat similar to *Siccus*-type broom cells, but with more short diverticula, ochraceous, abundant, main body 15–30 × 6–8 µm, some not branched, fusiform, thick-walled, trifurcate, bifurcate, more or less thin-walled, diverticulate. *Lamelar trama* dextrinoid, interwoven, hyaline, thin-walled. *Pileus* trama dextrinoid, interwoven, including the edge trama of the pileo, hyphae with clamp connections. *Pileipellis* hymeniform to irregularly hymeniform, cells often smooth all over, most bifurcates, afillate, some piriform, clavate, thick-walled, ochraceous brown to coppery brown, sometimes golden in the cells lumen, with hair 500 × 9 µm, brownish cooper, *Stipe trama* with parallel, cylindrical, brownish golden, thick-walled. *Clamp connection* present in all tissues, but the cells of the pileipelis. *Stipitipellis* with not differentiated cells.

Specimens Examined:—Brazil, Amazonas State, Manaus, Embrapa, 12 July. 1978, *R. Singer, Souza & Aguiar, B.11272* (Holotype, INPA82459); Reserva de Desenvolvimento Sustentável do Tupé, 06 September 2012, *D.L.Komura DLK917* (INPA270735).

Habit and Habita:—marasmioid, solitary on dicotyledonous leaf at Amazonian terra firme forest.

Distribution:—Brazil, Amazonas State.

Comments:— In the two collections examined no basidiospores were observed, but according to Singer (1989) *M. horridulus* basidiopores measure 7–8.8 × 3–4 µm, oblong, hyaline and inamyloid. *Marasmius calvocystidiatus* has similar basidiopores, but smaller 4.2–5.2 × 2.6–3.9 µm. *Marasmius horridulus* is recognized in the field as similar to *Crinipellis* because the hirsute pileus, but the lamellae are thick and attached to a collar, different in *Crinipellis* which presents adnate to free lamellae, and usually with lamellulae.

Phylogenetic analysis— The group formed by ITS analysis including *M. horridulus*, *M. calvocystidiatus* and *M. purpureisetosus* with high support (0.99 B-PP and 90 ML-BS) make some sense, once they share *Siccus*-type like broom cells with thick walled, lobate pileocystidia. *Marasmius horridulus* and *M. purpureisetosus* share the presence of hair-like structures on the pileus, however *M. horridulus* have long setae, around 500 × 9 μm, when compare with *M. purpureisetosus* setae 80–350 × 8–12 μm from Thailand (Wannathes et al. 2009).

The consistence of the pileus and stipe in *M. horridulus* and *M. calvocystidiatus* are more rigid when compare with *M. purpureisetosus* and also the number of lamellae are more numerous for the first two species (32–34 and 24 *vs* 13–17) (Wannathes et al. 2009).

According with molecular data, microscopic and macroscopic features we suggest that *M. calvocystidiatus* as a new species at subsection *Horriduli* within section *Marasmius* together with *M. horridulus*. However, Wannathes et al. (2009) describe the presence of pileosetae to *Marasmius purpureisetosus* and *M. berambutanus* just in this two species, neglecting the subsection *Horriduli* (Singer 1986, 1989). In fact, to a compheensive evolutive relationship for this group, more sequences and more samples are necessary.

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Legends of figures

FIGURE 1. Bayesian phylogram obtained from the ITS (ITS1-5.8S-ITS2) sequences. Support values (greater than 0.70) for major clades that are supported in either the Bayesian (posterior probabilities value–BPP) and maximum likelihood (bootstrap percentage–MLB) analyses are given above branches in bold. Scale bar value indicates nucleotide substitutions/site. Newly sequenced collections are in bold. *Chaetocalathus* spp. and *Crinipellis* spp. were used as outgroup.

FIGURE 2. *Marasmius calvocystidiatus* (DLK1516– Holotype). **a, d.** Basidiomata at leaf. **b,c.** Lamellae attached to collar. **b1,c1**. Detail of papilated pileus. **e.** Detail of the collar. **f.** Base of stipe and initial stage (black arrow). Scale bars: a= 5 mm. d= 10 mm.

FIGURE 3. *Maramius horridulus*. **a.** Basidiomata from Holotype. **a1.** Detail of the lamellae. **b.** Basidiomata (DLK917). **b1.** pileus with hairs. **b2.** Lamellae attached to collar. Scale bars: **a, b**= 3 mm.

FIGURE 4. *Marasmius calvocystidiatus* (DLK1516– HOLOTYPE). **a.** Tangential section through the pileus. **b.** close up tangential section through the pileus, in Melzer reagent. **c.** Cheilocystidia, **d.** Basidia layer with spores. **e.** Pileocystidia. **f.** Hyphae from context. **g.** Transversal section from the stipe. Scale bars: **c, e, f, g**= 20 μm.

FIGURE 5. *Marasmius calvocystidiatus* (DLK1516– HOLOTYPE). **a.** Basidiospores. **b.** Basidia and basidioles. **c.** Cheilocystidia. **d.** Pileocystidia. Scale bars= 10 μm. Illustrated by D.L.Komura.

FIGURE 6. *Marasmius horridulus*. **a.** Basidioles. **b.** Cheilocystidia. **c.** Pileocystidia. **d**, **e.** Hair from pileus. Scale bars= 10 μm. **e**= 100 μm. Illustrated by D.L.Komura.

TABLE 1. Strains and GenBank accessions of ITS sequences used in this study.

Fig. 1

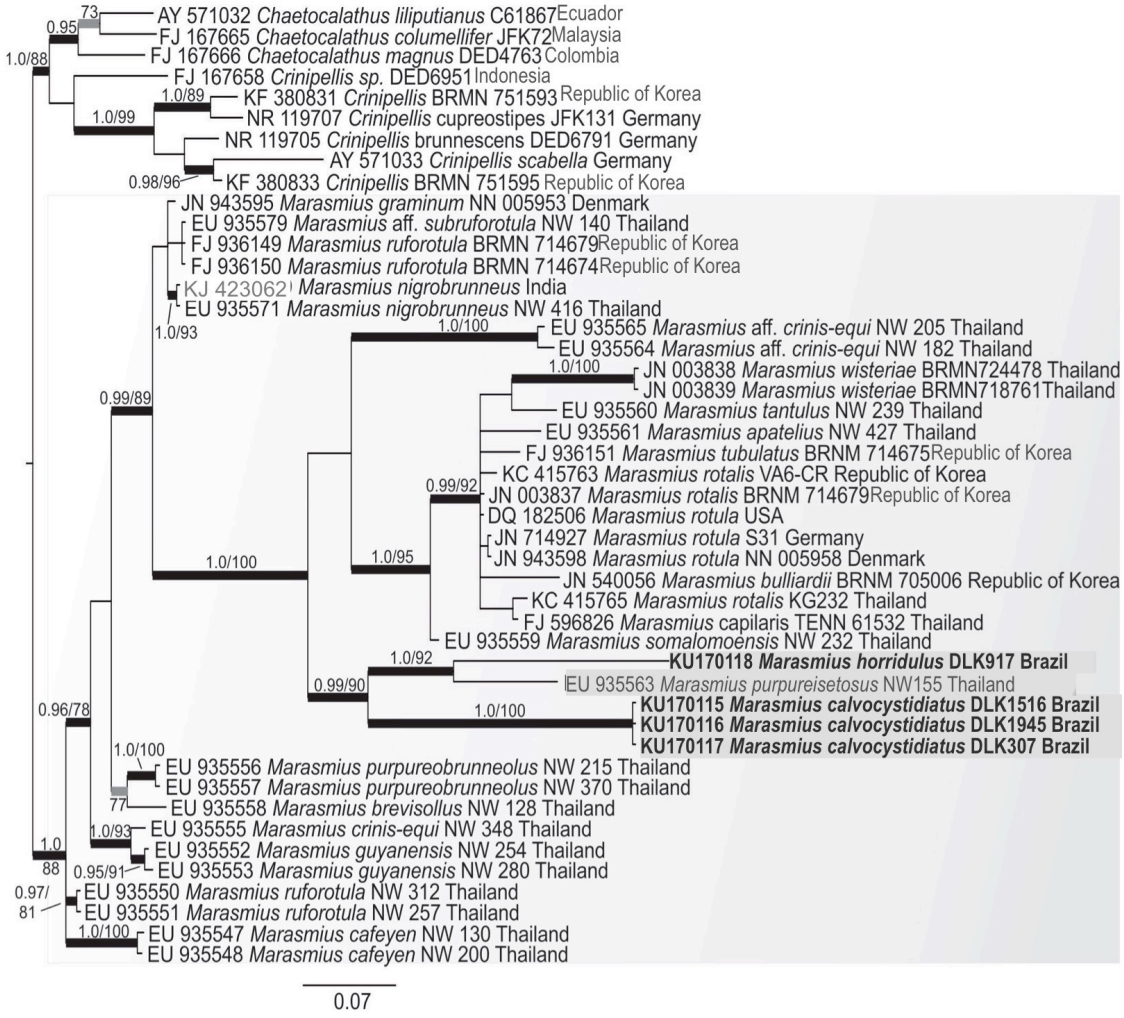
AY 571032 Chaetocalathus liliputianus C61867 Ecuador
FJ 167665 Chaetocalathus columellifer JFK72 Malaysia
FJ 167666 Chaetocalathus magnus DED4763 Colombia
FJ 167658 Crinipellis sp. DED6951 Indonesia
KF 380831 Crinipellis BRMN 751593 Republic of Korea
NR 119707 Crinipellis cupreostipes JFK131 Germany
NR 119705 Crinipellis brunnescens DED6791 Germany
AY 571033 Crinipellis scabella Germany
KF 380833 Crinipellis BRMN 751595 Republic of Korea
JN 943595 Marasmius graminum NN 005953 Denmark
EU 935579 Marasmius aff. subruforotula NW 140 Thailand
FJ 936149 Marasmius ruforotula BRMN 714679 Republic of Korea
FJ 936150 Marasmius ruforotula BRMN 714674 Republic of Korea
KJ 423062 Marasmius nigrobrunneus India
EU 935571 Marasmius nigrobrunneus NW 416 Thailand
EU 935565 Marasmius aff. crinis-equi NW 205 Thailand
EU 935564 Marasmius aff. crinis-equi NW 182 Thailand
JN 003838 Marasmius wisteriae BRMN724478 Thailand
JN 003839 Marasmius wisteriae BRMN718761 Thailand
EU 935560 Marasmius tantulus NW 239 Thailand
EU 935561 Marasmius apatelius NW 427 Thailand
FJ 936151 Marasmius tubulatus BRNM 714675 Republic of Korea
KC 415763 Marasmius rotalis VA6-CR Republic of Korea
JN 003837 Marasmius rotalis BRNM 714679 Republic of Korea
DQ 182506 Marasmius rotula USA
JN 714927 Marasmius rotula S31 Germany
JN 943598 Marasmius rotula NN 005958 Denmark
JN 540056 Marasmius bulliardii BRNM 705006 Republic of Korea
KC 415765 Marasmius rotalis KG232 Thailand
FJ 596826 Marasmius capilaris TENN 61532 Thailand
EU 935559 Marasmius somalomoensis NW 232 Thailand
KU170118 Marasmius horridulus DLK917 Brazil
EU 935563 Marasmius purpureisetosus NW155 Thailand
KU170115 Marasmius calvocystidiatus DLK1516 Brazil
KU170116 Marasmius calvocystidiatus DLK1945 Brazil
KU170117 Marasmius calvocystidiatus DLK307 Brazil
EU 935556 Marasmius purpureobrunneolus NW 215 Thailand
EU 935557 Marasmius purpureobrunneolus NW 370 Thailand
EU 935558 Marasmius brevisollus NW 128 Thailand
EU 935555 Marasmius crinis-equi NW 348 Thailand
EU 935552 Marasmius guyanensis NW 254 Thailand
EU 935553 Marasmius guyanensis NW 280 Thailand
EU 935550 Marasmius ruforotula NW 312 Thailand
EU 935551 Marasmius ruforotula NW 257 Thailand
EU 935547 Marasmius cafeyen NW 130 Thailand
EU 935548 Marasmius cafeyen NW 200 Thailand
0.07

Fig. 2 *Marasmius calvocystidiatus*

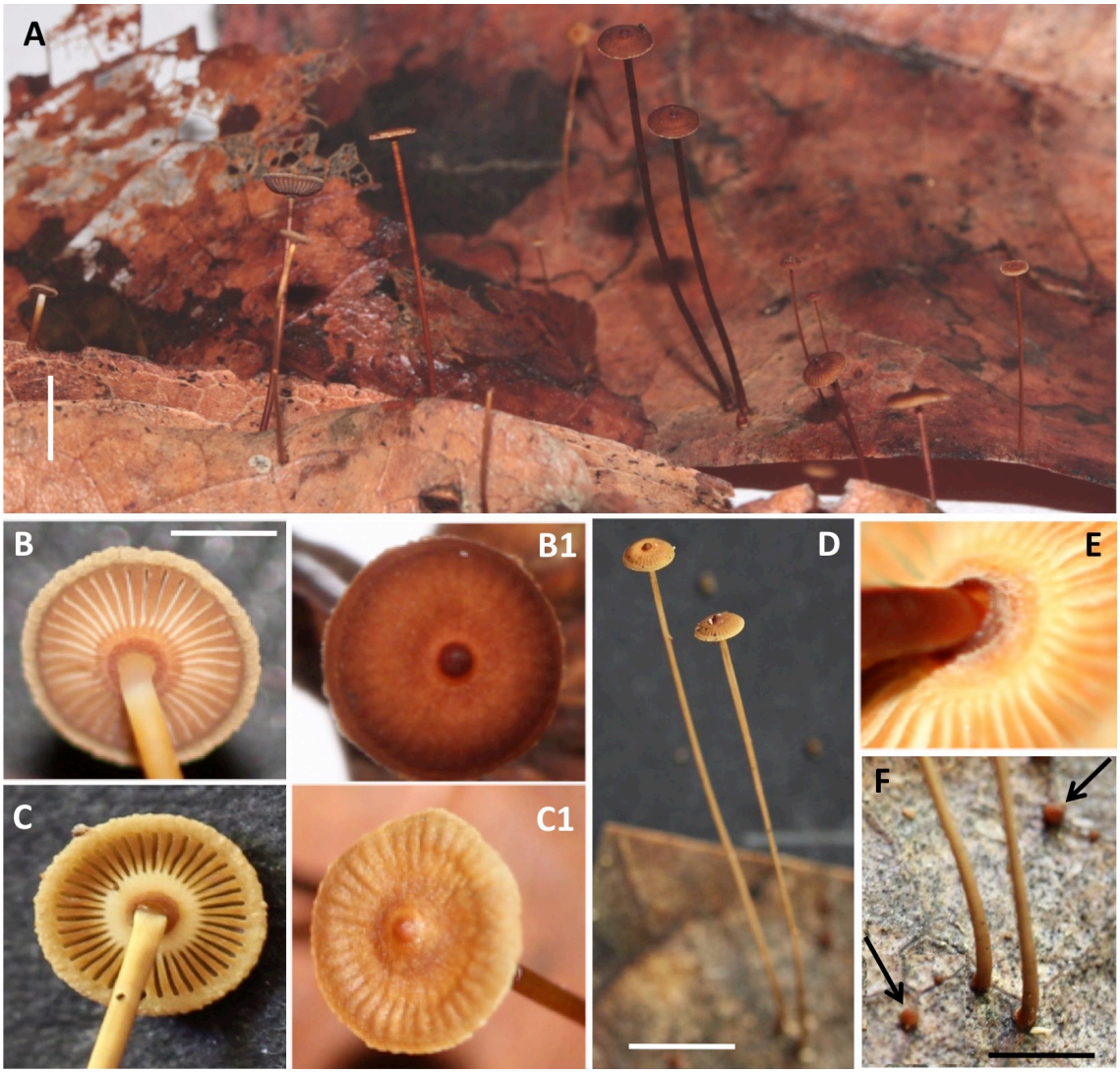

Fig. 3 *Marasmius horridulus*

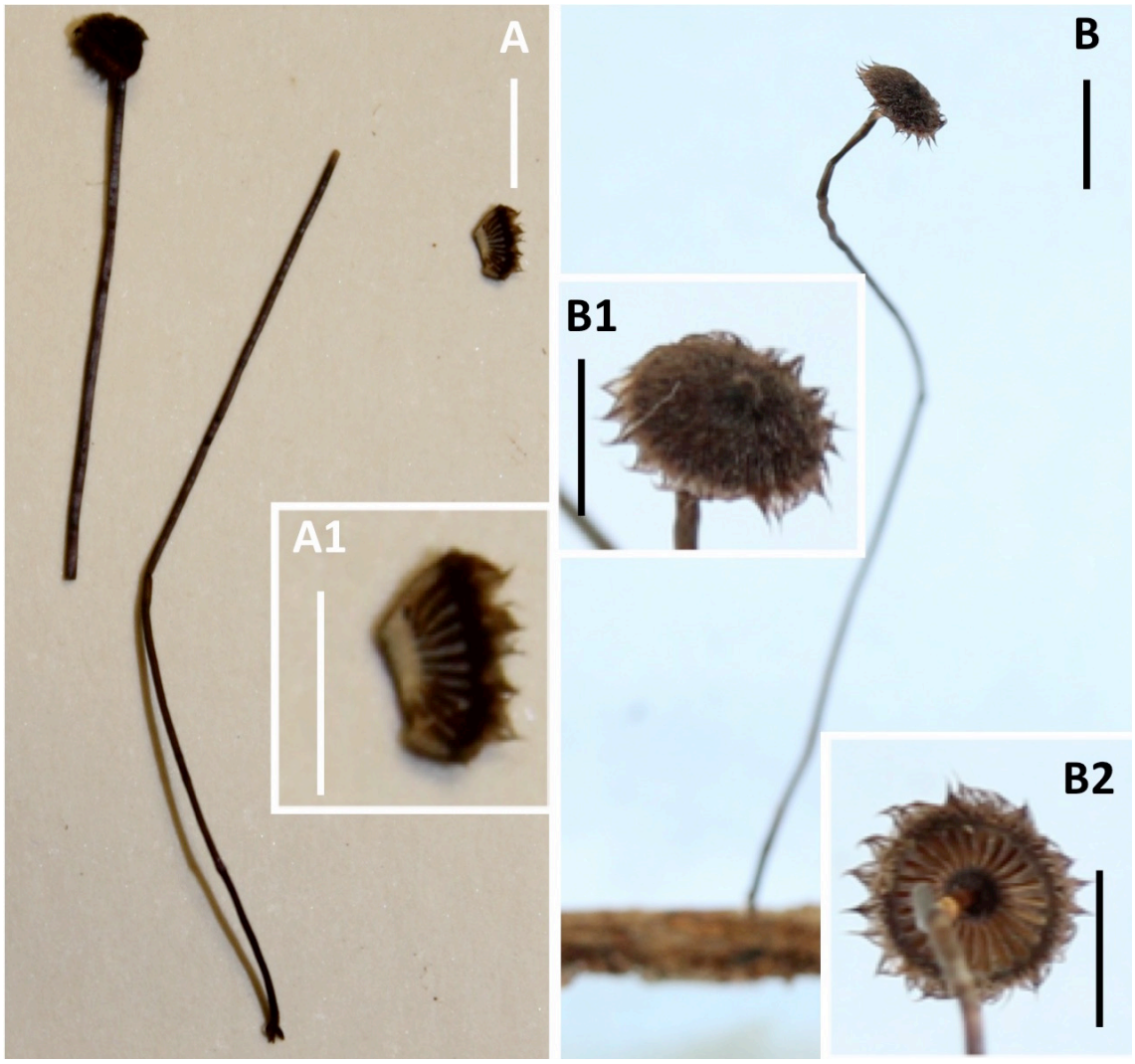

Fig. 4 *Marasmius calvocystidiatus*

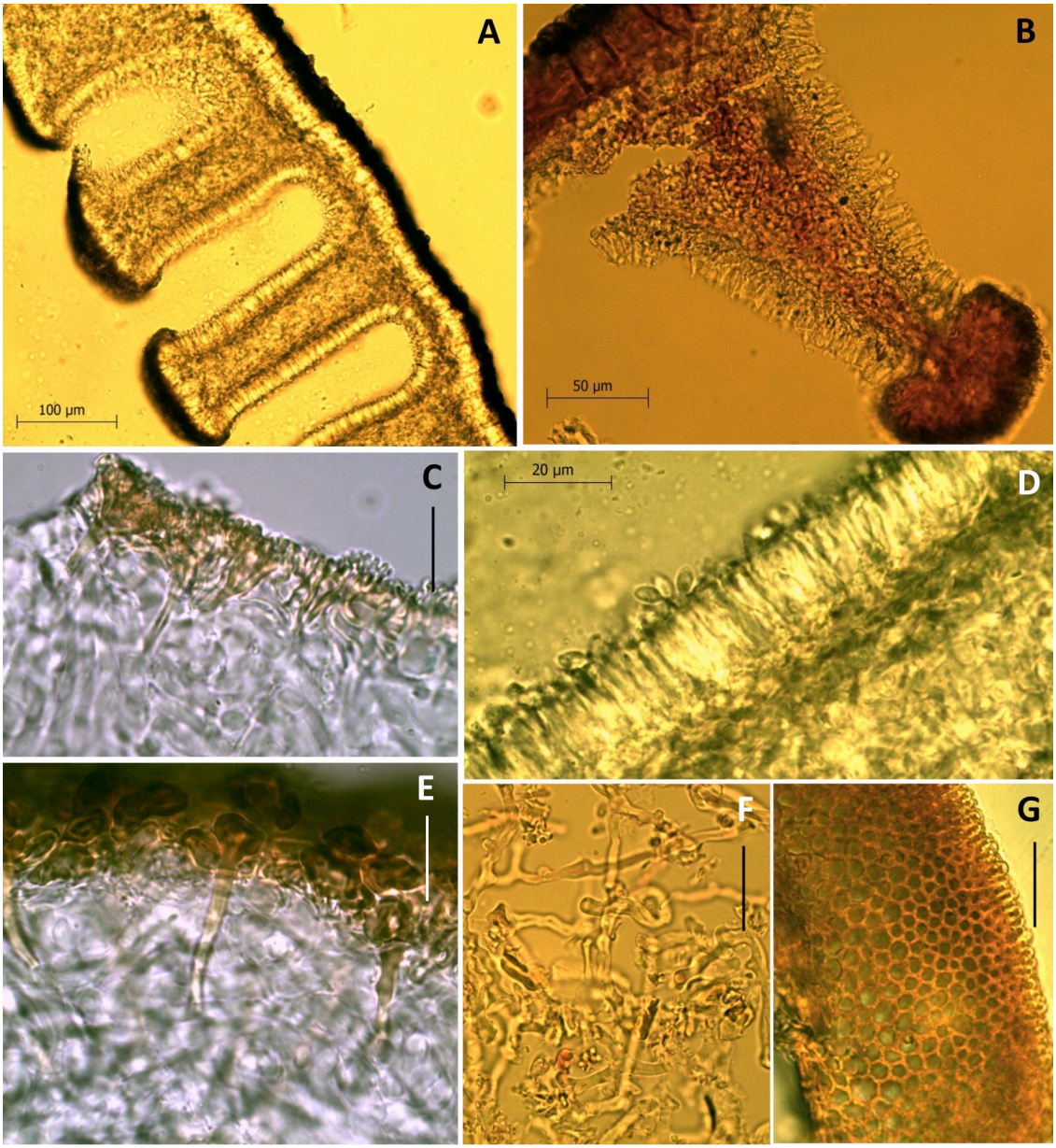

Fig. 5 *Marasmius calvocystidiatus*

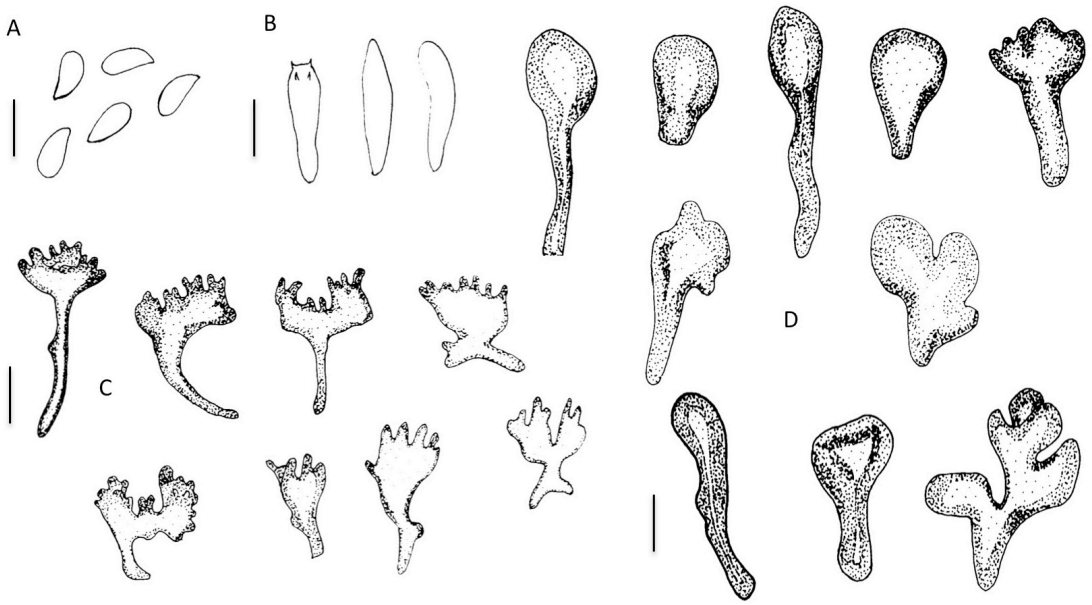

Fig. 6 *Marasmius horridulus*

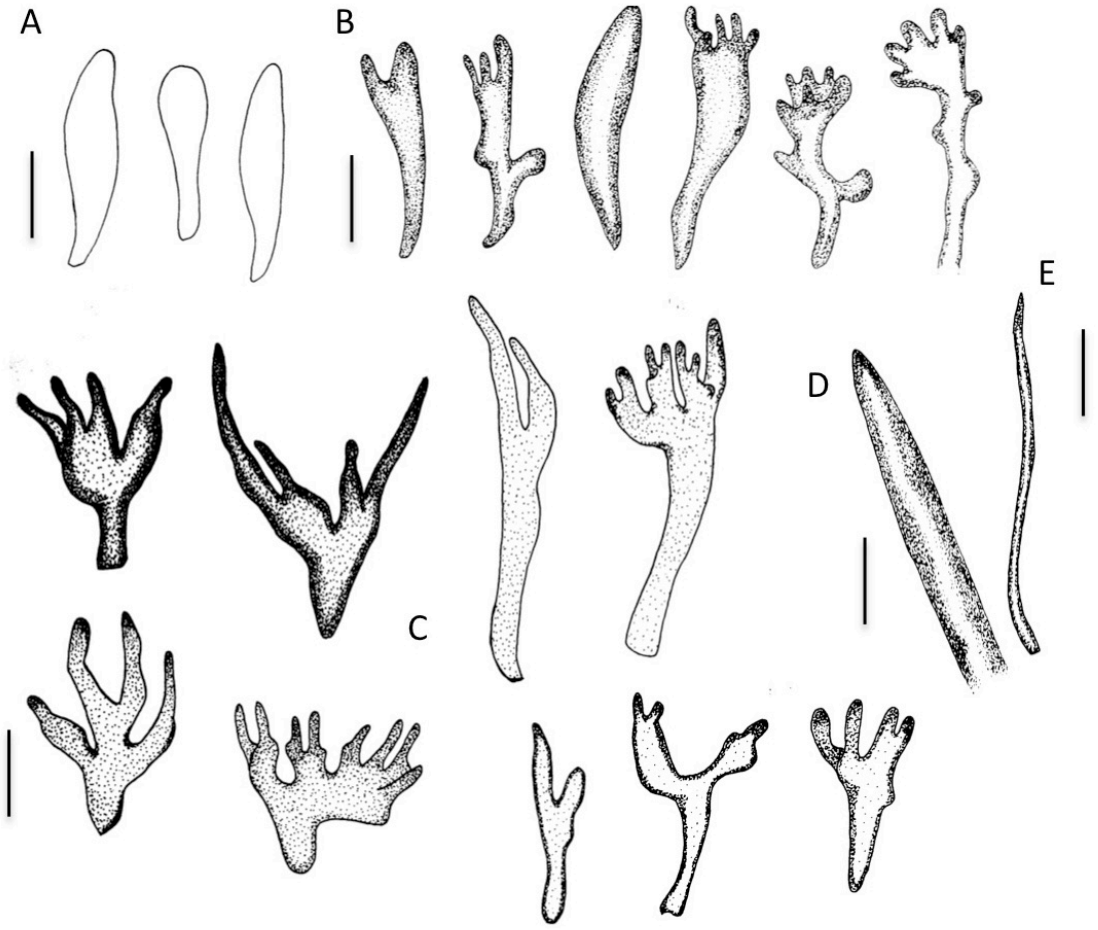

Table 1 Strains and GenBank accessions of ITS sequences used in this study.

Species	collection ID	Herbarium accession No.	Location	Genbank accession No.	Reference
Chaetocalathus columellifer (Berk.) Singer	JFK72		Malaysia	FJ167665	Kerekes & Desjardin 2009
Chaetocalathus liliputianus (Mont.) Singer	C61867		Ecuador	AY571032	Bodensteiner et al. 2004
Chaetocalathus magnus Halling		DED4763	Colombia	FJ167666	Kerekes & Desjardin 2009
Crinipellis sp.		BRMN 751593	Rep. Korea	KF380831	Ryoo et al. 2013, umpublished
Crinipellis sp.		BRMN 751595	Rep. Korea	KF380833	Ryoo et al. 2013, umpublished
Crinipellis brunnescens Kerekes & Desjardin		DED6791	Indonesia	NR119705	Kerekes & Desjardin 2009
Crinipellis cupreostipes Kerekes, Desjardin & Lumyong	JFK131		Thailand	NR119707	Kerekes & Desjardin 2009
Crinipellis scabella (Alb. & Schwein.) Murrill	PB302		Germany	AY571033	Bodensteiner et al. 2004
Crinipellis furcate Kerekes, Desjardin & Lumyong		DED6951	Indonesia	FJ167658	Kerekes & Desjardin 2009
M. aff. *crinis-equi*	NW205		Thailand	EU935565	Wannathes et al. 2009
M. aff. *crinis-equi*	NW182		Thailand	EU935564	Wannathes et al. 2009
M. aff. *subruforotula*	NW140		Thailand	EU935579	Wannathes et al. 2009
M. apatelius Singer	NW427		Thailand	EU935561	Wannathes et al. 2009
M. brevicollus Corner	NW128		Thailand	EU935558	Wannathes et al. 2009
M. bulliardii Quél.		BRNM705006	Rep. Korea	JN540056	Wannathes et al. 2009

M. cafeyen Wannathes, Desjardin & Lumyong	NW130		Thailand	EU935547	Wannathes et al. 2009
M. cafeyen	NW200		Thailand	EU935548	Wannathes et al. 2009
M. calvocystidiatus	DLK1516	INPA259372 (	Brazil	KU170115	this work
M. calvocystidiatus	DLK1945	INPA259374	Brazil	KU170116	this work
M. calvocystidiatus	DLK307	INPA270734	Brazil	KU170117	this work
M. capilaris Morgan		TENN61532	Thailand	FJ596826	Hughes et al. 2009
M. crinis-equi F. Muell. ex Kalchbr.	NW348		Thailand	EU935555	Wannathes et al. 2009
M. graminum (Lib.) Berk.		NN005953	Denmark	JN943595	Schoch et al. 2012
M. guyanensis Mont.	NW254		Thailand	EU935552	Wannathes et al. 2009
M. guyanensis	NW280		Thailand	EU935553	Wannathes et al. 2009
***M. horridulus* Singer**	DLK917	INPA270735	Brazil	KU170118	this work
M. nigrobrunneus (Pat.) Sacc.	CUHAM079		India	KJ423062	Dutta et al. 2014, Umpublished
M. nigrobrunneus	NW416		Thailand	EU935571	Wannathes et al. 2009
M. purpureobrunneolus Henn.	NW215		Thailand	EU935556	Wannathes et al. 2009
M. purpureobrunneolus	NW370		Thailand	EU935557	Wannathes et al. 2009
M. purpureisetosus Corner	NW155		Thailand	EU935563	Wannathes et al. 2009
M. rotalis Berk. & Broome	VA6-CR		Rep. Korea	KC415763	Antonin, Ryoo, Ka, Shin 2013
M. rotalis		BRNM724479	Rep. Korea	JN003837	Antonin, Ryoo, Ka, Shin 2013
M. rotalis	KG232		Thailand	KC415765	Wannathes et al. 2009
M. rotula (Scop.) Fr.			USA	DQ182506	Matheny, Hibbett 2005, Umpublished
M. rotula	S31		Germany	JN714927	Grobe et al. 2011

M. rotula	NN005958		Denmark	JN943598	Schoch et al. 2012
M. ruforotula Singer		BRNM 714679	Rep. Korea	FJ936149	Ryoo et al. 2009, Umpublished
M. ruforotula		BRMN 714674	Republic of Korea	FJ936150	Ryoo et al. 2009, Umpublished
M. ruforotula	NW312		Thailand	EU935550	Wannathes et al. 2009
M. ruforotula	NW257		Thailand	EU935551	Wannathes et al. 2009
M. somalomoensis Antonín	NW232		Thailand	EU935559	Wannathes et al. 2009
M. tantulus Wannathes, Desjardin & Lumyong	NW239		Thailand	EU935560	Wannathes et al. 2009
M. tubulatus Petch		BRNM714675	Rep. Korea	FJ936151	Ryoo et al. 2009, Umpublished
M. wisteriae Antonín, R. Ryoo & H.D. Shin		BRNM 724478	Rep. Korea	JN003838	Ryoo et al. 2009, Umpublished
M. wisteriae		BRMN718761	Rep. Korea	JN003839	Ryoo et al. 2009, Umpublished

Capítulo 4

Komura, D.L; Oliveira, J. J. S.; Moncalvo, J. M.; Margaritescu, S.; Zartman, C. E. Amazing ramified *Marasmius* from Amazon forest of Brazil.

Manuscrito formatado para a *Acta Amazonica*

Amazing ramified *Marasmius* from Amazon forest of Brazil

Dirce Leimi Komura[1*]; Jadson J. S. Oliveira[2;]; Jean-Marc Moncalvo[2]; Charles Eugene Zartman[1]

[1]Instituto Nacional de Pesquisas da Amazônia (INPA), Botany Dept., C.P. 478, 69011-970 Manaus, Amazonas, Brazil;

[2]Depart. of Natural History, Royal Ontario Museum, and Dept. of Ecology and Evolutionary Biology, University of Toronto, Canada;

* Corresponding author

Abstract

Marasmius with rhizomorphs from Brazilian Amazon are described, providing ITS data, macro and microscopic features.

Key words: *Marasmius cupressiformis*; *Marasmius microdendron*; *Marasmius populiformis*; rhizomorphs; taxonomy; Terra firme forest.

Introduction

Marasmius Fr. is a group of high species diversity, most of them saprotrophic and almost cosmopolitan, but at tropical areas we can find more number of species and abundance (Singer 1986, Antonín and Nooderloos 2010). In Neotropics, 233 species of *Marasmius* can be found (Singer 1976).

According to Kirk et al. (2008), around 500 species were described, while Wilson and Desjardin (2005) have estimated above one thousand species. At fungi repository database we may find 1640 names at MycoBank (http://www.mycobank.org), and 1946 names at Index Fungorum (http://www.indexfungorum.org) associated to *Marasmius*, including synonyms, varieties and invalid names.

All this divergence among number of species is because we still have few taxonomists to a huge, hyper diversity collection area as Amazonia. This fact we can observe, when check the number of *Marasmius* deposited at INPA herbaria from Manaus, Amazonas, Brazil (http://www.splink.org.br/index). Since, Singer and Araújo work's around 1978, the collection increment seems to go in pulses. After him, Souza, H.Q. (2001) and Braga-Neto (around 2005) and our collection in 2012 added an expressive collection and between this period just a sporadic deposits of *Marasmius* was recorded.

Beside that, the great morphological variation intra and inter-species are a challenge to identify those collections. However, the molecular data came to help to solve this problem and the identification based in morphological and molecular data, nowadays are indispensable. In relation to morphology features, here we presented rhizomorphs features, a structure usually found in *Marasmius* (Berkeley 1856, Singer 1976, 1989), but also in some species of *Gymnopus* [(as *Marasmius androsaceus* L.) Macdonald and Cartter 1961], *Marasmiellus* (Desjardin et al. 1993) and *Gloiocephala* (Singer 1976).

Rhizomorphs in *Marasmius* are completely absent in some taxa, could be determinant, as in *M. crinisequi, M. trichorhizus, M. zingiberianus*, but in some species are eventual or variable, as in *M. pallidocinctus var. latisporus, M. leucorotalis* (Oliveira 2014).

Rhizomorphs (literally, "root-forms") are structures in mushroom formed by linear aggregation of the hyphae. Moore (1994) describe in detail the structural morphology and development of this structure in fungi. In generally, are tubes with a central channel through the rhizomorph, and in mature tissues are composed by narrow-diameter, thick-walled fiber hyphae.

Jacques-Félix (1967 *apud* Hedge 1993) suggests that rhizomorphs represent stipes that elongation have persisted and while the basiodiomes have stalled. This was supported with in vitro experiment carried out by Gooday (1985) which marasmioid rhizomorph responded to gravity, light, carbon dioxide similar to basidiomycetes basidiomes (Hedger et al. 1993).

We can find some "types" of rhizomorphs, as telepods, aerial, creeping and erect. The term telepode, at first, was used to describe degraded or modified stipes to describe vegetative organs from which basidiomes arise in *Marasmius rotalis* Berk. & Br. and *M. rotula* (Scop.: Fr) Fr (Jacques-Félix 1967 *apud* Hedger et al. 1993). Singer (1976) use telepodes or "telepodia" to sterile stipes arising direct to the substrate, that accompany the basidiomes, usually thinner than stipes with pileus, could be glabrous, branched, mostly scanty. However many author do not make distinction between sterile and non-sterile rhizomorphs (MacDonald 1961, Desjardin et al. 1993).

The aerial rhizomorphs from marasmioid fungi are very common in tropical forest, those black hairs attached to the leaves and branches at canopy create a litter-trap produces a microhabitat (Hedger 1990). Snaddon et al. (2012) observed in an experiment, that the removal of this fungal litter-trap resulted in a decrease around 70% of arthropods morphospecies richness.

Marasmius crinisequi (horse-hair fungi) rhizomophs structure and development was described by Cairney (1991) and despite its importance as plant pathogenic, another study with tea bushes detected the emission a volatile compound causing defoliation by the fungi (Su et al. 2011), and the same time the fungi produces rhizomorph attaching to those leaves, keeping in the air, this strategy is advantageous to fungi, because avoid competition for substrate with other organisms on the soil (Hedger 1990; Su et al. 2011).

The marasmioid rhizomorphs are also found in bird nest in tropical forest (Singer 1986, Freymann 2008). Freymann (2008) observed that this material have a relatively high tensile strength and low water uptake, these features could bring advantages in the stability of the nest.

Another study with the ecological relation of the rhizomorph was conducted with southern flying squirrels nest in Alabama, USA, and was observed that 80% contained fungal rhizomorphs of *Marasmius brevipes*. This was the first description the relationship of fungi and a mammalian species (Prange and Nelson 2006).

Some *Marasmius* species could develop their basidiomes attached to an erect rhizomorph, seeming branched fungi. This feature common in tropical *Marasmius* is rare in Europe. Murril has proposed a genus for species with this habit, and Hennings a subgenus, but this is not a generic character nor does a hiatus exist between forms of this kind and other sections (Singer 1965). The branched basidiomes, as aerial or telepods rhizomorphs are not even diagnostic characteristic for some species and the same way of fruiting can be observed in related genera (Singer 1965).

Here we present some of these branched *Marasmius* from Amazon, providing ITS data, macro and micro characteristics.

Material and methods

Fieldwork

Specimens were collected during field expeditions along the years 2012–2015 in four sites in the Brazilian Amazon: (1) The Estação Experimental de Manejo Florestal of INPA (ZF-2) (02°37’ S, 60°09’ W), about 80 km north of Manaus, the state capital of Amazonas; (2) Reserva Biologica Ducke of INPA (2° 58' 48" S, 60° 09' 08" W) in the neighborhood of Manaus; (3) São Sebastião Community (2° 48' 04" S, 60° 29' 58" W), located at Cuieras river, a branch of Rio Negro river in Novo Airão and (4) Floresta Nacional do Tapajós (2° 51' 37" S, 54° 57' 57" W) at ICMBio Station Base Terra Rica, located at km 67 and at Jamaraquá Community (2° 49' 45" S, 55° 01' 56" W), Belterra, Pará State.

Morphological characterization

Macroscopics characteristics were described based on fresh material and/or photographs of that, before dried at ~ 40 °C. We used the color code based on Color Picker chart color (http://www.colorpicker.com). The microscopic observation was carried out according to Oliveira and Capelari (2012). Sections of dried material were rehydrated in 70% ethanol and mounted in 5% KOH or in Melzer reagent for the amyloidity test. The dimensions of the spore measurements included the range of length × width, and following statistical analysis: X_m, the arithmetic Mean of length × width ± standard deviation; Q_m, the mean of the range of length/ width of basidiospores ± standard deviation; and n, the number of spores measured. The lamellae spacing was determined by the following factors: L, the number of lamellae that reach from the stipe to the pileus margin; and l, the number of series of lamellulae among the lamellae. The dried collections were deposited in the INPA and duplicates were sent to Royal Ontario Museum Fungarium (TRTC).

ITS sequences production and analysis

DNA isolation, PCR amplification, sequencing and editing of the ITS region (ITS1-5.8S-ITS2) followed Dentinger et al. (2010). Sequences showing >90% similarity to the newly produced *Marasmius* sequences were retrieved from BLAST (Altshul et al. 1990) searches in the NCBI database (GenBank), and aligned with our sequences using MUSCLE (Edgar 2004). A preliminary analysis was conducted in MEGA version 6 (Tamura et al. 2013) using Maximum-Likelihood (ML) and default parameters. From this preliminary analysis (data not shown) we selected 35 ITS sequences (Table I) that were deemed to be relevant for this study. ML settings for the final analysis were determined in jModeltest 2.1.7 (Darriba et al. 2012). Bootstrap support (BS) for branches was estimated from 1,000 replications.

Results and discussion

Taxonomy

***Marasmius cupressiformis* Berk.**, Hooker's J. Bot. Kew Gard. Misc. 8: 140 (1856).

[MB#149212]

Holotype: K(M), Spruce n.75, 1853. **Type locality:** Brazil, Amazonas, Panuré.

Figures 2A and 2B

Pileus up to 2 mm diam., convex, sulcate, center slightly depressed with a acuminate, brown papilla, some very prominent, white (F5F9FA) even when dry, margin plane, surface glabrous, dry, dull; membranous, context very thin. *Lamellae* collariate, concolous with the pileus, few, *L*=8–10, smooth, edge regular. *Stipe* 2.5 × up to 0.2 mm, central, filiform, glabrous, bright, insititious, rising from central, erect, thicker rhizomorphs; apex whitish to cream, turning dark brown, more evident to the immature form; rhizomorphs 45- 50 × 1 mm, insititious, black with brown scars, likely spot of dropped stipe.

Basidiospores 6.5–8.1 × 3.3–4.6 µm [X_m= 7.3 ±0.4 × 4.0 ±0.3 µm; Q_m= 1.8 ±0.2; n=26], elliptic to obovoid, smooth, thin-walls, hyaline, inamyloid. *Basidia* 23.7 × 7.2, clavate, 4-

sterigmate. Basidiole 21 × 4.3, clavate, some acuminated. *Pleurocystidia* absent. *Cheilocystidia* of *Siccus*-type broom cells, similar to the pileipellis broom cells, main body 7–14 × 13–40 μm, irregular, branched, base thin, pedicellate, thick-walled, some walls very thick; diverticula (4 × 0.5 μm) digitiform, apex often obtuse, some acute, branched. *Lamellar trama* dextrinoid, irregular, hyphae cylindrical, smooth, thin-walled. *Pileus* trama dextrinoid, irregular, narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid; *pileocystidia* two principal type (1) resemble to *Siccus*-type (10–20 × 4–15), some thin-walled and some thick walled, branched, diverticulate and (2) somewhat *Rotalis*-type (12–15 × 8–10), some with fingers-like projections and also some cystidias bulboid and pedunculate, lampiform, thick-walled.

Habit and Habitat: marasmioid on erect rhizomorphs, close, leaves of dicotyledonous tree in Amazonian terra firme forest.

Distribution: Brazil in the state of Amazonas; Zaire, Singer (1976) suggests as Pantropical.

Specimens Examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 26 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1398 (INPA270739); 23 May 2013. D.L.Komura & O.F. Menezes, DLK1527 (INPA259378).

Comments:

This species was collected by Spruce at Brazilian Amazonia on Alto Rio Negro, Uaupés ("Panuré") and described by Berkely in 1856, after that, it was collected and identified by Singer in 1978 in Manaus. Singer (1964) also described this species from Congo, and then he suggests that this species could be Pantropical. Now we recollected this species and its macroscopic analysis are according to Singer description (1976).

The presence of pileocystidia similar to *Siccus*- and *Rotalis*-type broom cells are characteristics that distinguish this species in relation to others with similar macromorphology. Singer (1965) also describes the presence of *Chrysochaetes*-type cells,

probably were referring to the thick-walled cells that also we have found. However, in his later publication he does not use this term to describe the collection.

Marasmius cupressiformis are very similar to collection DLK823, also collected in the same locality, but differ in the glabrous rhizomorphs and the pileus even dried maintained its whitish color, differing the DLK823 when dried the pileus turning cream to brownish and also is more fragile than *M. cupressiformis*.

Marasmius* aff. *cupressiformis

Figures 3A

Pileus 2–3 mm diam., convex, sulcate, center slightly depressed with a acuminate, brown papilla, white (F5F9FA) becoming cream (FAEEBB) when dry, margin plane, very thin context; *Lamellae* collariate, concolous with the pileus, few, *L*=9–10, thin and smooth. *Stipe* 4–5 × < 0.2 mm, central, cylindrical thin, circular, apex whitish to cream, turning light brown, more evident to the immature form; with hyaline and short hairs; rhizomorph 50–60 × 1 mm, with whitish cream short hairs, more dense than stipe. Basidiomes concentrated at apex of an erect rhizomorph. The basidiomes formation not have an organization along the rhizomorphs, we can find different stages of basidiomes mixed.

Basidiospores 7 × 4 µm (not found enough to statistic data), elliptics to obovoid, smooth, thin-walls, hyaline, inamyloid. *Basidia* clavate, 4-sterigmate. *Pleurocystidia* absent. *Cheilocystidia* horny, some with short diverticula, branched, bifurcated, base thin, pedicellate, ochreaceous, main body 11–21 × 14–5 µm, diverticules 2–6 µm, obtuse, finger-like, similar to the pileipelis broom cells. *Lamelar trama* dextinoid, irregular. *Pileus* trama dextrinoid, interwoven, very narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid, *Pleurocystidia* main body 9–24 × 6–16 µm, cells often smooth all over, sometimes irregular and lobed, mostly regular when clavate, pyriform, some long pedicellate, deepening in the pileus trama, thick-walled, ochreaceous brown to coppery brown, sometimes golden in the

cells lumen. *Stipe trama* with parallel, cylindrical, brownish golden, thick-walled. *Clamp connection* present in all tissues, but the cells of the pileipelis. *Stipitipellis* with hairs, pale brown to yellowish, thick-walled. *Rhizomorph* similar to the stipitipellis structures.

Habit and Habitat: marasmioid, rhizomorphs on leaf of dicotyledoneous tree on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Specimens Examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 27 Jun. 2012, D.L.Komura, DLK823 (INPA270740).

Comments:

This species differ from the *M. cupressiformis*, regarding the characteristics cited before, the cystidia here are more lobate and diverticulate, not resemble to *Siccus* or *Rotalis* type. *Marasmius* aff. *cupressiformis* is also related *M. multiceps*, color and shape of the pileus, but this have large pileus 12- 14 mm *vs.* up to 3 mm diam. *Marasmius* aff. *cupressiformis* have hyaline, hirsute stipe and rhizomorphs while *M. cupressiformis*, *M. multiceps* and *M. submulticeps* are glabrous. In addition, in these two later species the rhizomorph are creeping differing to our specimen here that have an erect, and the stipe form a racemose structure.

Marasmius microdendron Singer, Sydowia 18: 349 (1965).

[MB#333732]

Holotype: BAFC, R. Singer (B3448), 1960. **Type locality:** Brazil, Amazonas, 30 km N of Manaus.

Figures 4A and 4B

Pileus up to 1 mm diam., plane campanulate to broadly convex, sulcate, pink (FC68A6), center slightly depressed with a chestnut brown to pale brown papila (784631), margin incurved to plane, edge glabrous, dry, opaque concolous with the pileus; *Lamellae* collariate,

colar thick, concolous with the pileus edge, few, *L*=7–10, white, thick. *Stipe* ~2 × 0.1 mm, central, filiform, smooth, glabrous, bright; apex whitish to cream and then brown (7A442C), attached by a volva-like node to rhizomorph 150 × 1 mm, reddish brown (7D3719) at apex to black, smooth, polish, flexible. The basidiomes are concentrated at apex of the erect rhizomorph.

Basidiospores 5.3–7.6 × 2.1–3.8 µm [X_m= 6.6 ±0.5 × 3.8 ±0.5 µm; Q_m= 2.1 ±0.2; n=15], elliptics to obovoid, smooth, thin-walls, hyaline, inamyloid. Basidia 21 × 7 µm, 4-sterigmate, clavate. Basidioles, 21–16 × 6–5 µm, clavate, afilate. *Pleurocystidia* absent. *Cheilocystidia* somewhat similar to *Siccus*-type broom cells, lumen purple in KOH, dextrinoid in Melzer, main body 17–9 × 4–11 µm, diverticulate, lobate, branched, thick-walled diverticula 2- 6 µm, obtuse, finger-like. *Lamelar trama* dextrinoid, irregular. *Pileus* trama dextrinoid, interwoven, including the edge trama of the pileo, hyphae with clamp connections. *Pileipellis* hymeniform to irregularly hymeniform, cells often smooth all over. Pileocystidia similar to cheilocystidia but with also pedicelate, claviform, pyriform, thick-walled, golden cells, mixed on the pileus, and more concentrated at papilla. *Stipitipellis* without diferentiated structures.

Habit and Habitat: marasmioid, rhizomophs on leaf of dicotyledonous tree on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Pará

Specimens Examined: Brazil, Pará State, Belterra, ICMBio Base Terra Rica, km 67, 28 Mar. 2014, D.L.Komura & T.S. Cabral, DLK1938 (INPA270741).

Comments:

This is a very difficulty species to identify. Oliveira (2014) summarize the species close related. The pilear surface of *M. microdendron* is similar those described to *M. edwallianus*, *M. hippiochaetes, M. minusculus* and *M. polycladus* with purple pigmented cells observed in KOH preparation (Singer 1976).

Marasmius edwallianus is very close related, but have larger basidiospores (7–8 × 3–

4.2 μm) and pileus 0.8–2.5 mm broad and smooth stipe and rhizomorph. We examined the *M. microdendron* holotype, and observed that stipe and rhizomorph were glabrous, differing to its original description, this characteristic placed our specimen more related to *M. microdendron*, however the insertion of the stipe to rhizomorphs showed a volva-like base is describe to *M. polycladus* Montagne. But this species have a creeping rhizomorph, instead an erect, dendroid-ascendant form similar *M. hyppiochates*, *M. edwallianus* and *M. microdendron*.

***Marasmius populiformis* Berk.**, *Hooker's J. Bot. Kew Gard. Misc.* **8**: 140 (1856).

[MB#524333]

Holotype: ?, Spruce s.n.,1853. **Type locality:** Brazil, Amazonas, Panuré.

Figures 5A and 5B

Pileus ~1 mm diam., plane campanulate to broadly convex, sulcate, brown (8C4D32), center slightly depressed, pale with a black papila, margin incurved, dry, opaque concolous with the pileus; *Lamellae* collariate, colar thick, concolous with the depressed part of the pileus, few, *L*=7–9, pale, thick. *Stipe* ~2–10 × 0.1 mm, irregular length, central, filiform, smooth, glabrous, bright; golden (FACF6B), attached to rhizomorph 60 × 1 mm, golden (FACF6B), smooth, polide, flexible. The basidiomes are concentrated at apex of the erect rhizomorph. In the same collection we found a basidiome with stipe insert direct to the substrate.

Basidiospores 7.8–8.0 × 3.9–4.0 μm [X_m= 7.7 ±0.5 × 3.9 ±0.3 μm; Q_m= 2.0 ±0.2; n=6], elliptic to oblong, smooth, thin-walls, hyaline, inamyloid. *Basidia* 20 × 8, clavate, 4-sterigmate. *Pleurocystidia* absent. *Cheilocystidia Rotalis*-type broom cells, similar to the pileipelis broom cells 15 × 17 μm, thin-walled. *Lamelar trama* dextrinoid, irregular, hyphae cylindrical, smooth, thin-walled. *Pileus* trama dextrinoid, irregular, narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid; *pileocystidia Rotalis*-type broom some thick-walled.

Habit and Habitat: marasmioid, rhizomorph on dicotyledonea branch on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Amazonas and Bahia

Specimens Examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1195 (INPA270742); 24 May 2013, D.L.Komura & O.F.Menezes, DLK1581 (INPA270743); ReBio Ducke, 30 May 2013, D.L.Komura, T.H.G. Oliveira, J.G.A.Nascimento & A.T.Melo, DLK1596 (INPA259380).

Comments:

This species is one of the tiniest pileus, is easy to recognize for the golden yellow, polished stipe and rhizomorph. The pileipelis are the *Rotalis*-type broom cells.

M. populiformis also was described by Berkeley for the same locality to *M. cupressiformis*, in Amazonia. In 1978, Singer identified this species from Manaus-Amazonas and Porto Seguro-Bahia, suggesting that this species occur in both Amazonian and Atlantic forest.

***Marasmius* sp. 1**

Figures 6A and 6B

Pileus 5–7 mm diam., plane convex to convex, sulcate, center slightly depressed, with a pale zone around a small brown dot, fulvous red (E33702) orangish red FC6B3F) to deep reddish orange (DB3C0B), margin incurved, edge entire to wavy; surface glabrous, dry, dull, subvelutinous; membranous, context very thin. *Lamellae* collariate, cream (FFFAD1), few, *L*=10–14, smooth, edge regular finely concolous with the pileus, collar edge orangish red (E33702). *Stipe* 5–10 mm × up to 0.2 mm, central, filiform, bright; apex whitish to light brown (BA6C45), becoming dark brown, with reddish brown (A33C08) hairs, scarce, more present at the base; rhizomorph 35–60 × 1 mm, rigid, erect, hirsute-pilose, densely covered by the reddish brown hairs; some thinner, creeping rhizomorphs growing attached to the substrate.

Basidiospores 13 × 4 μm, (not found enough to statistic data), oblongo, clavate to subfusoid, smooth, thin-walls, hyaline, inamyloid. Basidia not observed. Basidiolo 30 × 7, clavate, afilate *Pleurocystidia* absent. *Cheilocystidia* in form of *Siccus*-type broom cells, similar to the broom cells of the pileipelis, main body 10–22 × 5- 10 μm, clavate, turbinate, forked, and thin-walled, clamp connections present at the base, setulae apical, erect 2–4 μm, cylindrical or digitiform, hyaline, regular, apex obtuse to acute. *Lamellar trama* dextrinoid, irregular, hyphae smooth, hyaline, above 3 μm, clamped. *Pileus* irregular, hyphae smooth, hyaline, above 3 μm, clamped, similar to lamellar trama. *Pileipellis* hymeniform, dextrinoid, composed by *Siccus*-type broom cells, 5–18 × 3–8 μm cylindrical to turbinate, largely varying in size, many irregular in outline, thin-walled, clamp connections present at the base; setulae apical erect, short to moderate in length 2–5 μm, digitiform to cylindrical, few conical to verruciform, regular, apex obtuse to acute. *Stipe trama* dextrinoid. *Stipitipellis* (also the rhizomorph surface) covered by hairs which are elongated, slander, 4 μm diam, brownish to ocher, filiform, rising laterally from cortical hyphae layer from the stipe, thick-walled septated.

Habit and Habitat: marasmioid, on dicotyledonous leaf on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Specimens Examined: Brazil, Amazonas State, Novo Airão, Comunidade São Sebastião - Rio Cuierias, 13 Apr. 2014, D.L.Komura 2010 (INPA259377).

***Marasmius* sp. 2**

Figures 7A and 7B

Pileus ~1 mm diam., plane campanulate to broadly convex, sulcate, brown (61321A), center slightly depressed, pale with a cream papilla, margin incurved, dry, opaque concolous with the pileus; *Lamellae* collariate, collar thick, concolous with the pileus, few, *L*=10–12, pale,

thick. *Stipe* ~2 × 0.1, central, filiform, smooth, glabrous, bright; reddish brown (944822), attached to rhizomorph 150 × 1 mm, brown (9E6A51), smooth, polish, horny. The basidiomes are concentrated from the middle to apex of the erect rhizomorph.

Basidiospores not observed. *Basidia* 24 × 7, clavate, 4-sterigmate. Basidiole 25 × 7, clavate. *Pleurocystidia* absent. *Cheilocystidia* irregular, branched, lobate, some without any projection, most thick-walled with amber lumen, main body 14–35 × 4–14 μm; diverticula (4 × 0.5 μm) digitiform, apex often obtuse, some acute, branched. *Lamelar trama* dextrinoid, irregular, hyphae cylindrical, smooth, thin-walled. *Pileus* trama dextrinoid, irregular, narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid; *pileocystidia* similar to cheilocystidia.

Habit and Habitat: marasmioid, rhizomorphs on dicotyledonea branches on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Specimens Examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 Aug. 2012, D.L.Komura, DLK885 (INPA 259379).

Comments: The ITS sequences for this specemen were not available. This specimen are great similarity, especial those macroscopics with *M. eucladopus* described from Bolivia (Singer 1965, 1976). However, to more conclusive identify we need to check the basidiospores and the presence of the "*Rotalis*-type broom cells which, in the majority covered down to the middle by diverging short setulae, or reminiscent of the *Siccus*-type" as decribed by Singer (1976). Here at first inspection, the broom cells here is neither *Siccus* and nor *Rotalis*-type. *Marasmius misionensis* presented more similarity to our cystidia describing as *Siccus*-type but transient to the *Chrysochaetes*-type, but the lamellae differ in having white or whitish edge, distant (up to 7), while our speciemen have lamellae edge concolor with pileus and number of lamellae (10-12).

***Marasmius* sp. 3**

Figures 8A and 8B

Pileus 5–10 mm diam., broadly convex to plane, slightly sulcate, some uplift to tuberculate striate, center depressed discolor with pileus, no papilla, orange red (BF3111), surface glabrous, dry, dull; membranous, context thin. *Lamellae* collariate, orange red (BF3111) in mature basidiome, for young, the collar is very tight to stipe and disconcolous with the pileus, cream (F5EED5), few, *L*=12–13, smooth, edge regular, concolor with the pileus. *Stipe* variable × up to 0.3 mm, central, filiform, glabrous, bright, insititious, rising rhizomorphs from nodes; apex whitish to cream, turning dark brown. *Rhizomorph* with more than one node until the stipe reaches pileus. Just one branch was observed.

Basidiospores 7.2–8.0 × 3.5–4.0 µm [X_m= 7.2 ±0.8 × 5.0 ±0.3 µm; Q_m= 2.0 ±0.2; n=4], elliptic to obovoid, smooth, thin-walls, hyaline, inamyloid. *Basidia* 25 × 6, clavate, 4-sterigmate. Basidiole 20–25 × 5–6, clavate, some acuminated. *Pleurocystidia* absent. *Cheilocystidia Siccus*-type broom cells, similar to the pileipellis broom cells, main body 12–20 × 7–9 µm, base thin, pedicellate, thin-walled; diverticula (0.5 × 4 µm) digitiform, apex often obtuse, some acute, branched. *Lamellar trama* dextrinoid, irregular, hyphae cylindrical, smooth, thin-walled. *Pileus* trama dextrinoid, irregular, narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid; *pileocystidia* of *Siccus*-type (9–20 × 5–10), most thick-walled, branched, diverticulate; diverticula (0.5–5), often acute, some obtuse. *Stipe* with no differentiated structures.

Habit and Habitat: marasmioid on erect rhizomorphs, close, on dicotyledonea leaf on leaf litter at Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Specimens Examined: Brazil, Pará State, Belterra, Floresta Nacional do Tapajós, Comunidade Jamaraquá, 24 Marc. 2014, D.L.Komura, I.R. Fonseca & T.S. Cabral, DLK1855 (INPA270744); DLK1856 (INPA270745).

Comments: This species is similar to description of *M. robertsonii* (Singer 1976), especially about the stipe and rhizophorph features, also the microscopic descriptions more or less similar.

***Marasmius* sp. 4**

Figures 9A and 9B

Pileus 6–11 mm diam., convex and plane and wavy when mature, sulcate, center depressed, discolor, with a brown papilla, red wine (A10D32) to purple in old basidiome (590419), margin wavy, surface glabrous, dry, velvet, in young specimen pileus margin concolous with lamellae; membranous, context thin. *Lamellae* collariate, concolous with the pileus, narrow, few, *L*=11–13, smooth to velvet, edge regular, peach color (FCA38B). *Stipe* 25–60 × up to 0.8 mm, central, filiform, glabrous, bright, insititious, rising direct from substrate or from node of erect, rhizomorphs; apex whitish to cream, turning dark brown, more evident to the immature form; rhizomorphs 25 × 0.8 mm, insititious, not branched.

Basidiospores not observed. *Basidia* 30 × 10, clavate, 4-sterigmate. *Basidiole* 22–40 × 5- 6, clavate, some acuminated. *Pleurocystidia* setula-like, afilate, some clavate, lumen light purple, some golden, thick-walled, main body 35–60 × 5–10 µm, wall 2- 4 µm thick. *Cheilocystidia*, branched, bi- tri-forked, clavate, irregular, thick walled, main body 6–12 × 23–30 µm; branches, acute, some obtuse, (3–8 µm). *Pileus* trama dextrinoid, irregular, interwoven, thick, hyphae smooth, clamped, around 4 µm. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid; *pileocystidia* three principal type (1) broom cells similar to cheilocystidia, irregular, branched, bi- tri- or more forked, think walled, main body 35–60 × 5–10 µm; branches acute, some obtuse, (3–8 µm), (2) some are similar to pleurocystidia, setiformi, clavate, pedunculate (20–50 × 5–10), (3) few similar in form to the two type decribed before, but with lumen light purple and thick-walled, golden. *Stipe* with no differentiated cells.

Habit and Habitat: marasmioid on erect rhizomorphs, on dicotyledonea leaf on leaf litter in Amazonian terra firme forest.

Distribution: Brazil, Amazonas.

Specimens Examined: Brazil, Amazonas State, Novo Airão, CIAPA, 16 Apr. 2014, D.L.Komura & T.S. Cabral, DLK2049 (INPA270747); DLK2050 (INPA 259746).

Comments: This species, differ to other branched species in form just a simplex branch arising froma scale. The microscopic feature is unusual in the *Marasmius*, with setiform, thick walled pleurocystidia. The macroscopic descripition about the pileus color and shape, and lamellar color is similar to *M. panerythus*, but Singer (1976) just mentions umbilicate center, while our specimen this area is paling and with small papilla. The description of pleurociystia and also the epicuticular elements is more or less similar to our sample.

***Marasmius* sp. 5**

10A and 10B

Pileus 2–4 mm diam., convex, sulcate, center slightly depressed, discolor, with an acuminate, brown papilla, some very prominent, orange (E85D13) to red (913300), margin plane, surface glabrous, dry, dull; membranous, context very thin. *Lamellae* collariate, concolous with the pileus, distant, *L*=11–12, smooth, edge regular. *Stipe* 2–6 × up to 0.2 mm, central, filiform, glabrous, bright, insititious, rising from nodes of central, erect, thicker rhizomorphs; apex whitish to cream, turning dark brown, more evident to the immature form; rhizomorphs 30–70 × 0.5 mm, insititious, black with nodes, branches irregular.

Basidiospores 8.0–9.0 × 4.0–5.0 μm [X_m= 8.4 ±0.5 × 4.8 ±0.4 μm; Q_m= 1.8 ±0.2; n=4], elliptic to obovoid, smooth, thin-walls, hyaline, inamyloid. *Basidia* not observed. Basidiole 15 × 5, clavate. *Pleurocystidia* absent. *Cheilocystidia Siccus*-type broom cells, similar to the pileipellis broom cells, main body 8–18 × 5–10 μm, irregular, branched, base thin, pedicellate, thin-walled; diverticula (0.5 × 5 μm) digitiform, apex often obtuse, some acute, branched.

Lamellar trama dextrinoid, irregular, hyphae cylindrical, smooth, thin-walled. *Pileus* trama dextrinoid, irregular, narrow. *Pileipellis* hymeniform to irregularly hymeniform, dextrinoid,; *pileocystidia* of *Siccus*- type broom cells, similar to cheilocystidia, thick-walled, main body 10–20 × 5–8 μm; diverticula (0.5 × 5 μm) digitiform, apex often obtuse, some acute, branched. *Stipe* with no differentiated cells.

Habit and Habitat: marasmioid on erect rhizomorphs or insert direct to the susbtrate, on dicotyledonea leaf on leaf litter in Amazonian terra firme forest.

Distribution: Brazil, Amazonas and Pará State.

Specimens Examined: Brazil, Pará State, Belterra, Floresta Nacional do Tapajós, Comunidade Jamaraquá, 24 Mar. 2014, D.L.Komura, I.R. Fonseca & T.S. Cabral, DLK1874 (INPA270748); Amazonas State, Novo Airão, CIAPA, 16 Apr. 2014, D.L.Komura, Aurélio & T.S. Cabral DLK2043 (INPA 259747); DLK2044 (INPA270749).

ITS sequences analysis

ITS analysis of the *Marasmius* sequences are very difficult due their very variable bases sequences and length, here the variation were from 498 to 739 bases. Despite that, the ITS analysis was usufull to species delimitation, most of them with high support.

Marasmius populiformis in this work, is the unique species that have *Rotalis*-type broom cells and are more related with others species with the same type broom cells with high support (BS 95%), which do not form rhizomorph.

Other groups with high support (BS 98%) are composed by species with orange reddish pileus and *Siccus*-type broom cells. This group showed not related with another group with the same type broom cells.

Other species we can see a molecular delimitation corroborating with morphological characters to distinguing the species. *Marasmius* aff. *cupressiformis* is distinct from *M. cupressiformis*, this last one is more related to *M. microdendon* and *M. polycladus* with

moderate support (87% BS). *Marasmius* aff. *cupressiformis* are close related with *Marasmius* "branched5" (not described in this study) with moderate support (88% BS). These three species have in commnon broom cells that resemble to *Siccus*-type, but also thick-walled and pigmented cells.

Due the limited sequences analysed, just one region of DNA, we cannot infer more about the evolutionary relationship associated with morphogical characters. In fact, the presence of rhizomorph is not a good character to infrageneric delimitation. The specimens described here with rizomorph have low molecular similarity with the sequences analysed, this alied with the modified broom cells character, do these specimens good candidates to incluiding in more comprehensive phylogenetic studies with the aim to understanding the infrageneric relationship, once the Neotropics specimens still not included in this studies.

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Legends

Tab. 1- Strains and GenBank accessions of ITS sequences used in this study

Fig.1- Maximum likelihood obtained from the ITS (ITS1-5.8S-ITS2) sequences data showing relationship among branched *Marasmius*. Bootstrap percentage (BS) greater than 70% are given along branches. Scale bar value indicates nucleotide substitutions/site. Newly sequenced collections are in bold.

Fig.2A- *Marasmius cupressiformis.* A- Basidiome on rhizomorph; B- Detail of lamellae attached to collar; C- Top of the pileus, showing brown papilla; D- Detail of the branches, some without pileus; E- Irregular development of branches and the pileus showing mature and immature pileus. Scale bar: 10 mm.

Fig.2B- Microscopic features of *Marasmius cupressiformis*. A- Basidiospores; B- Transversal view, showing hymenium, pileus trama and pileipellis (left to right); C, D, E, F- Detail of the pileocystidia; G- Pileocystidia from view from the top. Scale bar: 10 μm.

Fig 3A- *Marasmius* aff. *cupressiformis*. A- Basidiome on rhizomorph; B- Detail of the top of the pileus, showing mature and immature forms, differences on the papilla; C- Lamellae

attached to collar; D- Stipe and rhizomorph with hyaline hairs, and stages of basidomes development. Scale bars: A=10 mm, C= 3 mm.

Fig 3B- Microscopic features of *Marasmius* aff. *cupressiformis*. A- Basidiospores; B, C- *Siccus*-type cystidia; D- Hairs presents at stipitipelis and rhizomorph. Scale bars: A, C, D= 10 µm.

Fig. 4A- *Marasmius microdendron*. A- Basidiomes attached on the top of an rhizomorph; B- Detail of the pileus, stipe rising from volva-like from rizomorph, and stages of basidiomes development; C- Pileus; D- lamellae attached to collar. Scale bar- 20 mm.

Fig. 4B- Microscopic features of *Marasmius microdendron*. A- Basidiospores; B- Tangencial view of the pileus; C, D, E- Pileocystidia with purple color. Scale bars- A, C, D, E= 10 µm; B= 20 µm.

Fig. 5A- *Marasmius populiformis*. A- Basidiomes attached to rhizomorph; B- Close up of the pileus; C- Lamellae attached to collar. Scale bar- 15 mm.

Fig. 5B- Microscopic features of *Marasmius populiformis*. A, B- Transversal view of the lamellae and pileus in Melzer, lamellar trama dextrinoid (redish color on the middle); C- Pileocystidia view of the top; D- Pileocystidia *Rotalis*-type. Scale bars- A, B= 100 µm, C= 40 µm, D= 20 µm.

Fig. 6A- *Marasmius* sp.1. A- Basidiomes rising from rhizomoph. B- Detail of the pileus; C- Lamellae attached to a collar; D, E, F- Close up of the basidiomes; G- Rhizomorphs creeping on the leaf. Scale bar- 15 mm.

Fig. 6B- Microscopic features of *Marasmius* sp.1. A- Basidiospores; B, C- Pileocystidia *Siccus*-type; D- Cheilocystidia *Siccus*-type and basidioles; E- Brownish hairs rising from parallel cell of the rhizomorph. Scale bars-10 µm.

Fig. 7A- *Marasmius* sp.2. A- Basidiomes attached to erect rhizomorph; B- Detail of the pileus; C- Overview of the entire fungi; D- Close up of the pileus; E- Detail the stipe rising

from a nodes from rhizomorph; F- Detail of the very thick lamellae attached to collar; G- View from down side of the pileus. Scale bar- 20 mm.

Fig 7B- Microscopic features of *Marasmius* sp.2. A- Pileocystidia; B, D- Cheilocystidia; C- stipitipellis. Scale bar- 30 μm.

Fig. 8A- *Marasmius* sp.3. A- Basidiomes. B- Lamellae attached to collar of immature pileus; C- Pileus, depressed on the centre; D- Lamellae of mature pileus, lateral view; E- Lamellae attached to collar of mature pileus. Scale bar- 15 mm.

Fig. 8B- Microscopic features of *Marasmius* sp.3. A- Cheilocystidia *Siccus*-type; B, C, D- Pileuscistidia *Siccus*-type. Scale bar- 15 μm.

Fig. 9A- *Marasmius* sp. 4. A- Basidiomes, attached to rhizomorph (right) and simplex form; B- mature and elder (left) pileus; C- young pileus; D- lamellae view mature basidiome; E- detail of a young basidiome growing from the leaf; F- detail of the node where from rising the stipe; G- lamellae attached to collar young basidiome; H- lamellae view.

Fig. 9B- *Marasmius* sp. 4. Microscopic features. A- transversal cutting of the pileus, pileipellis (top), interwoven trama, hymenium; B- pleurocystidia; C- detail of the hymenium with pleurocystida (setiform and purplish cells); D- Pileipelis. E- “golden” type pileocystidia. F- tri-forked pileocystidia; G, H- Pleurocystidia. Scale bar- 20 μm.

Fig. 10A- *Marasmius* sp. 5. A- Basidiomes attached to the top of rhizomorph; B- Irregular pattern of branched where the basidiomes rinsing from; C- Lamellae attached to a collar; D- pileus detail.

Fig. 10B- Microscopic features of *Marasmius* sp. 5. A- Basidiospores; B, E- Pileocystidia; C, D- Cheilocystidia. Scale bar- 10 μm.

Table 1 Strains and GenBank accessions of ITS sequences used in this study.

Species	collection ID	Herbarium accession No.	Location	Genbank accession No.	References
M. capillaris Morgan		TENN61532	USA	FJ596827	Hughes et al. 2009
M. capillaris		TENN61533	USA	FJ596826	Hughes et al. 2009
M. capillaris		TENN61534	USA	FJ596828	Hughes et al. 2009
M. capilaris		TENN61532	Thailand	FJ596826	Hughes et al. 2009
M. guyanensis Mont.	NW254		Thailand	EU935552	Wannathes et al. 2009
M. guyanensis	NW280		Thailand	EU935553	Wannathes et al. 2009
M. rotalis Berk. & Broome	VA6-CR		Rep. Korea	KC415763	Antonin et al. 2013
M. rotalis		BRNM724479	Rep. Korea	JN003837	Antonin et al. 2013
M. rotula (Scop.) Fr.	S31		Germany	JN714927	Grobe et al. 2011
M. rotula	NN005958		Denmark	JN943598	Schoch et al. 2012
M. ruforotula Singer	NW312		Thailand	EU935550	Wannathes et al. 2009
M. ruforotula	NW257		Thailand	EU935551	Wannathes et al. 2009
M. crinis-equi F. Muell. ex Kalchbr.	TYS338		Malaysia	FJ431233	Tan et al. 2009
M. crinis-equi	TY341		Malaysia	FJ431232	Tan et al. 2009
M. crinis-equi	TY466		Malaysia	FJ431231	Tan et al. 2009
*M.*cf. *suthepensis*	DLK1844	INPA271966	Brazil		this work
M. leucorotalis Singer	TYS489		Malaysia	FJ431253	Tan et al. 2009
M. tubulatus Petch	TYS502		Malaysia	FJ431280	Tan et al. 2009
M. tubulatus	TYS490		Malaysia	FJ431281	Tan et al. 2009
M. tantulus Wannathes, Desjardin & Lumyong	NW239		Thailand	EU935560	Wannatheset al. 2009
M. populiformis	DLK1596	INPA259380	Brazil		this work
M. rotula	PAN279		Poland	KM085384	Trocha;Rudy 2014
M. rotula		NN005958	Denmark	JN943598	Schoch et al. 2011
Gymnopus androsaceus (L.) J.L. Mata & R.H. Petersen	TFB4720		Sweden	DQ444315	Mata et al. 2006
Gymnopus androsaceus	TFB4702		Sweden	DQ444313	Mata et al. 2006
Gymnopus androsaceus	TFB3745		UK	DQ444312	Mata et al. 2006
Marasmius sp.3	DLK1855	INPA270744	Brazil		this work
Marasmius sp.1	DLK2010	INPA259377	Brazil		this work
Marasmius sp.5	DLK2043	INPA259747	Brazil		this work
Marasmius sp.5	DLK1874	INPA250748	Brazil		this work
Marasmius aff. *cupressiformis*	DLK823	INPA270740	Brazil		this work
Marasmius branched5	DLK838		Brazil		this work
M. cupressiformis	DLK1398	INPA270739	Brazil		this work
M. cupressiformis	DLK1527	INPA259378	Brazil		this work
M. microdendron	DLK1938	INPA270741	Brazil		this work
M. polycladus Mont.	JO418		Brazil		Oliveira 2014
M. polycladus	JO423		Brazil		Oliveira 2014

Fig.1- Maximum likelihood obtained from the ITS (ITS1-5.8S-ITS2) sequences data showing relationship among branched *Marasmius*

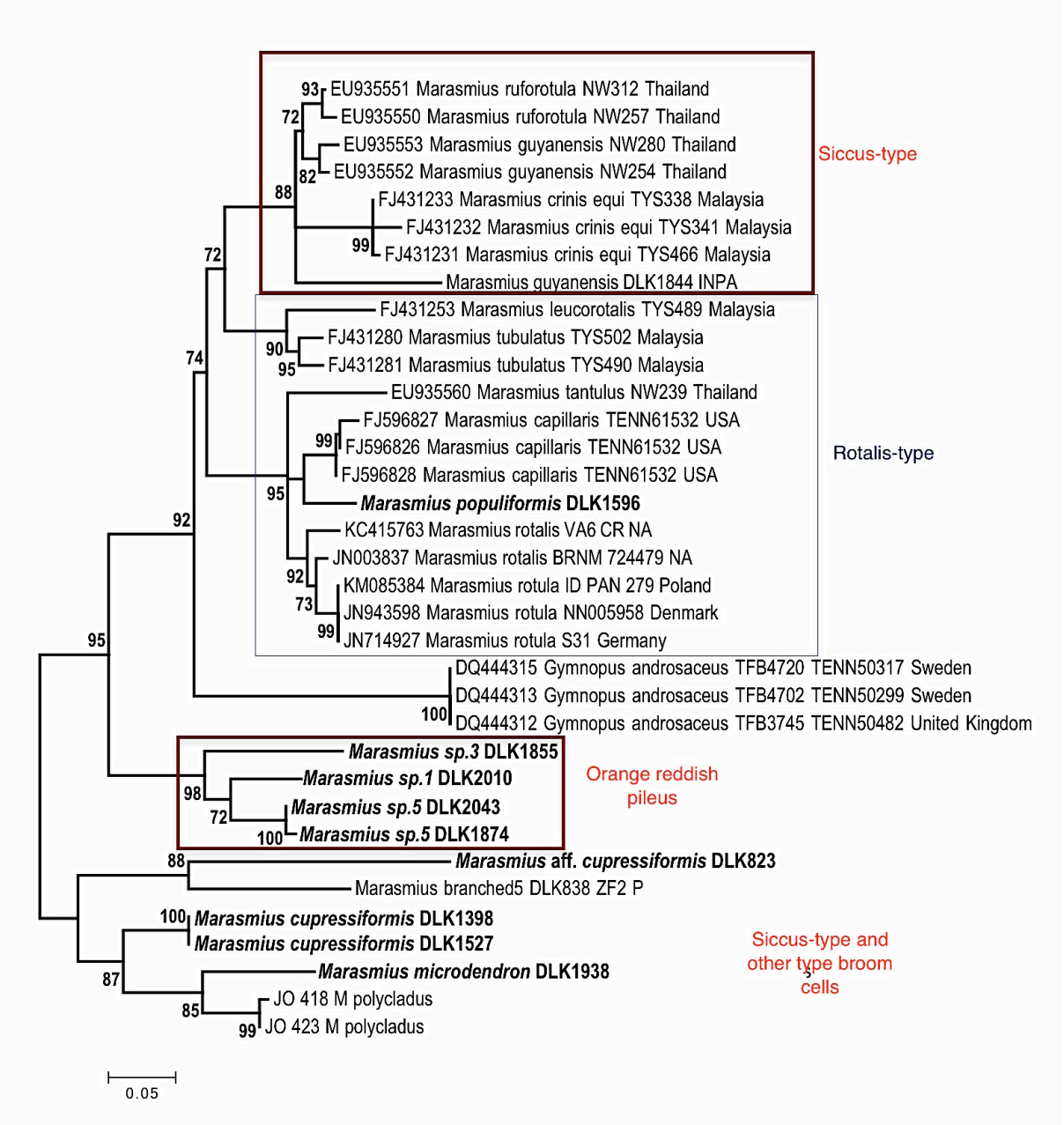


Fig.2A- *Marasmius cupressiformis*

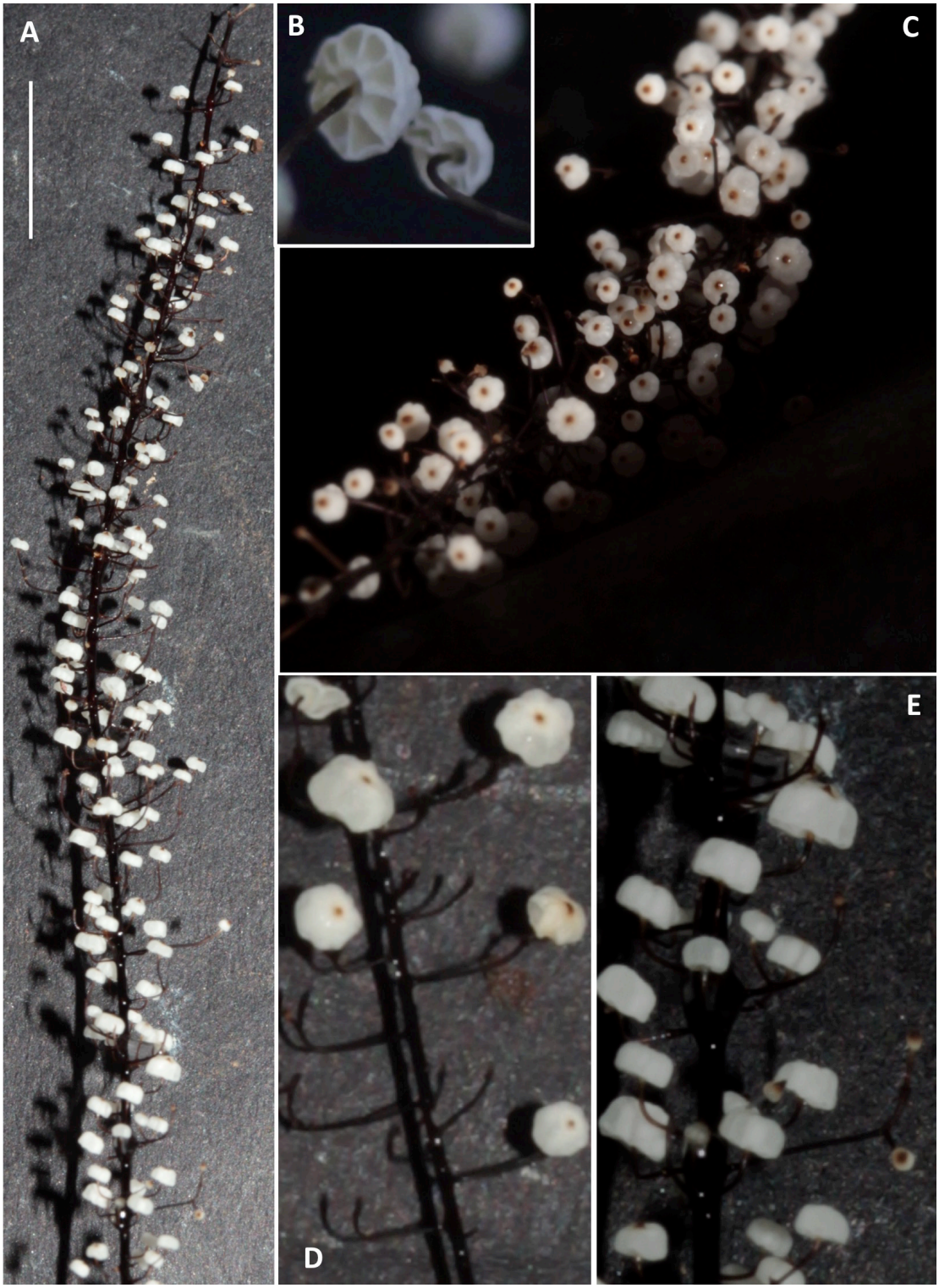


Fig.2B- *Marasmius cupressiformis*

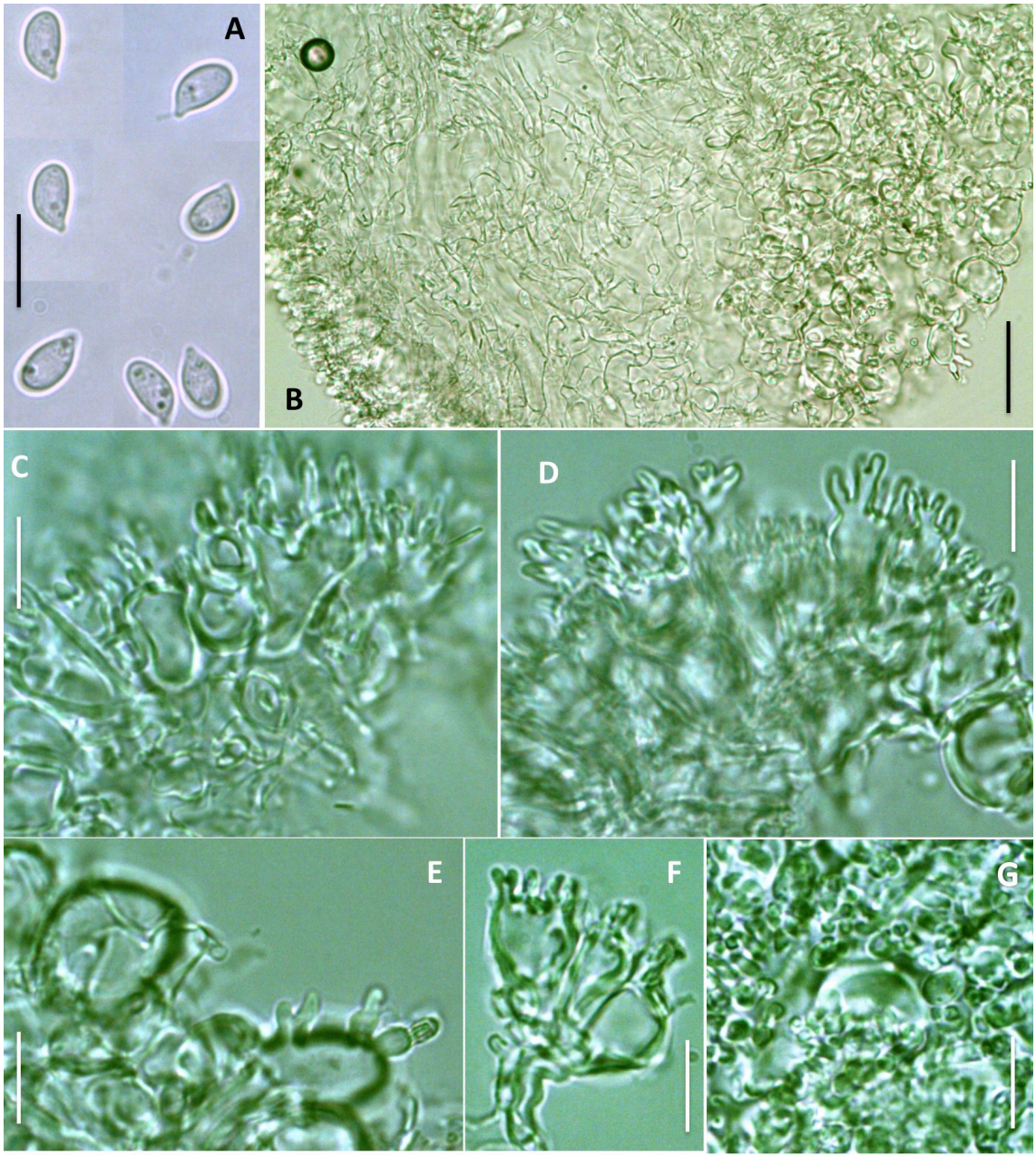


Fig.3A- *Marasmius* aff. *cupressiformis*

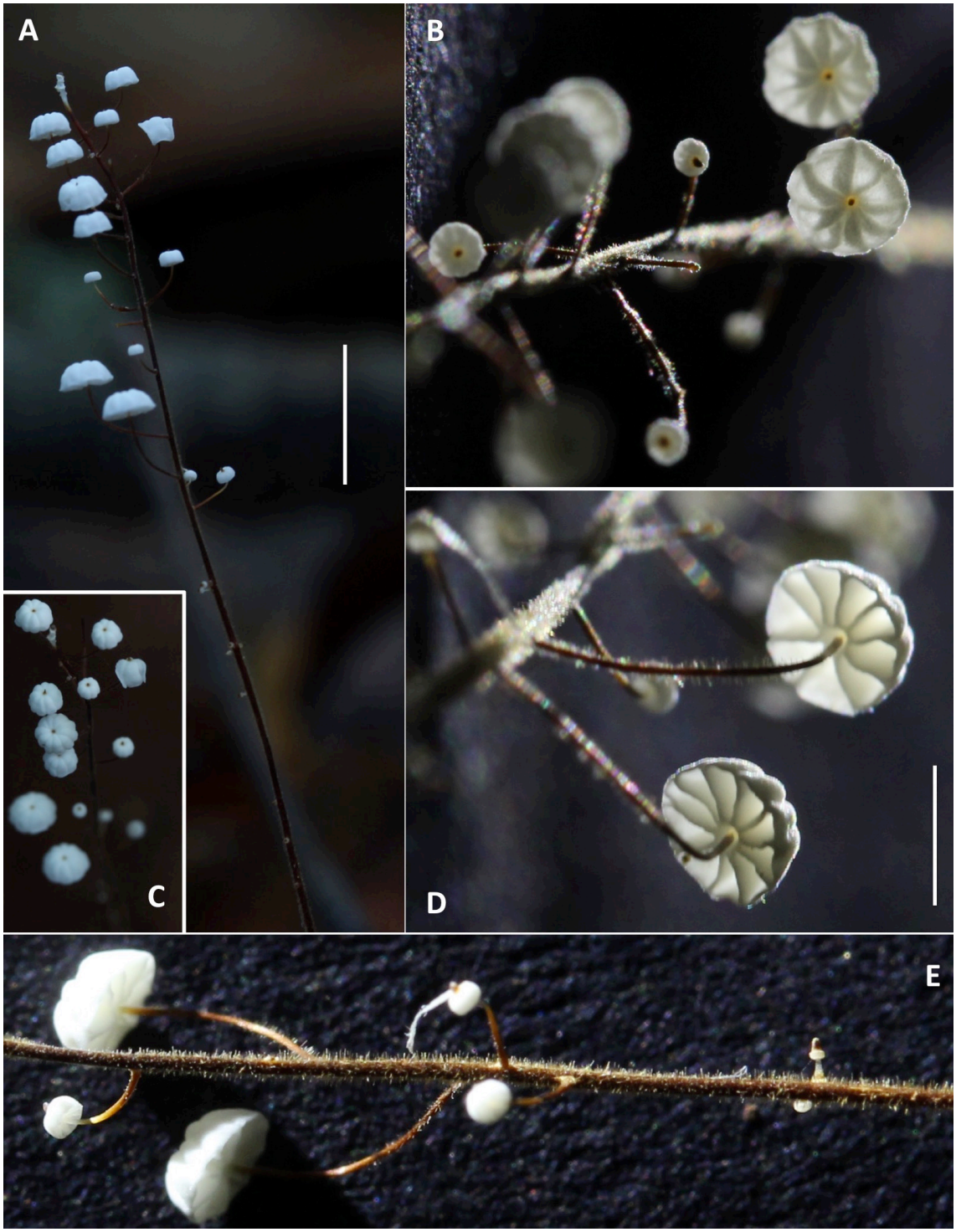


Fig.3B

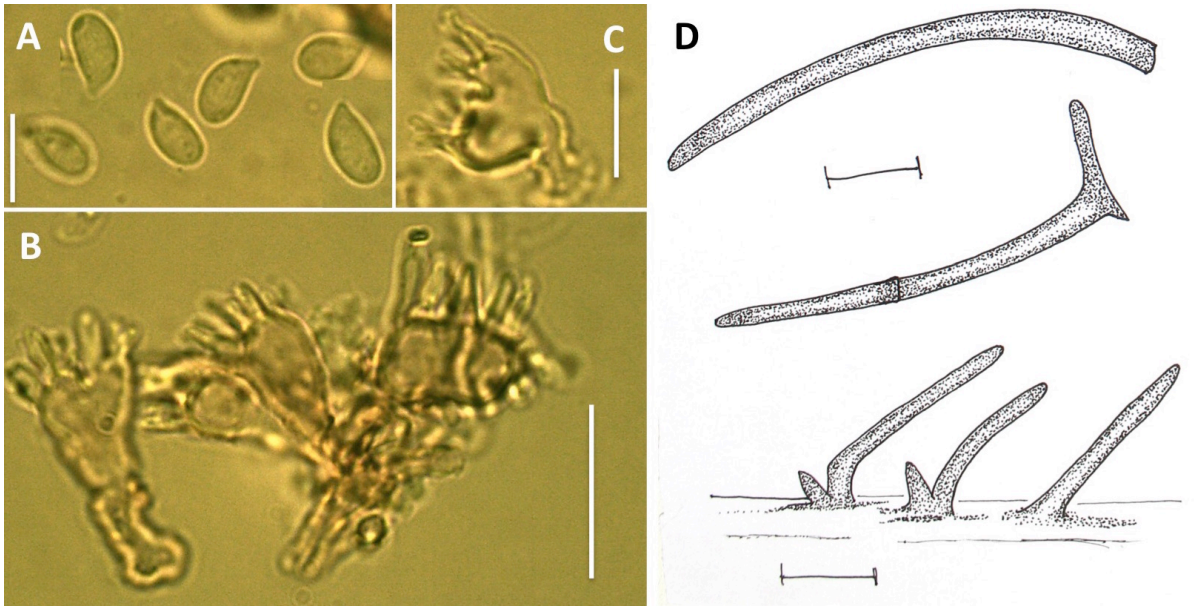


Fig.4A- *Marasmius microdendron*

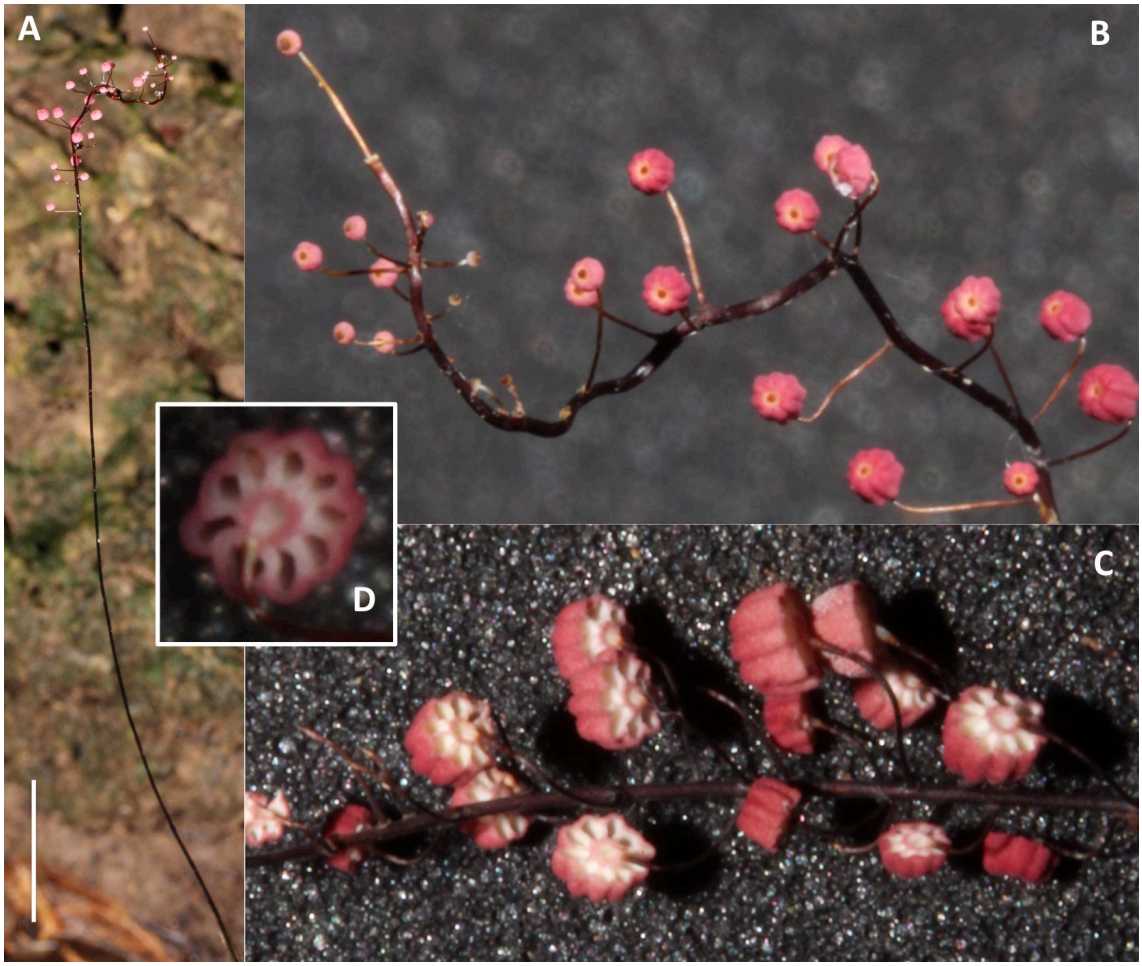


Fig.4B- *Marasmius microdendron*

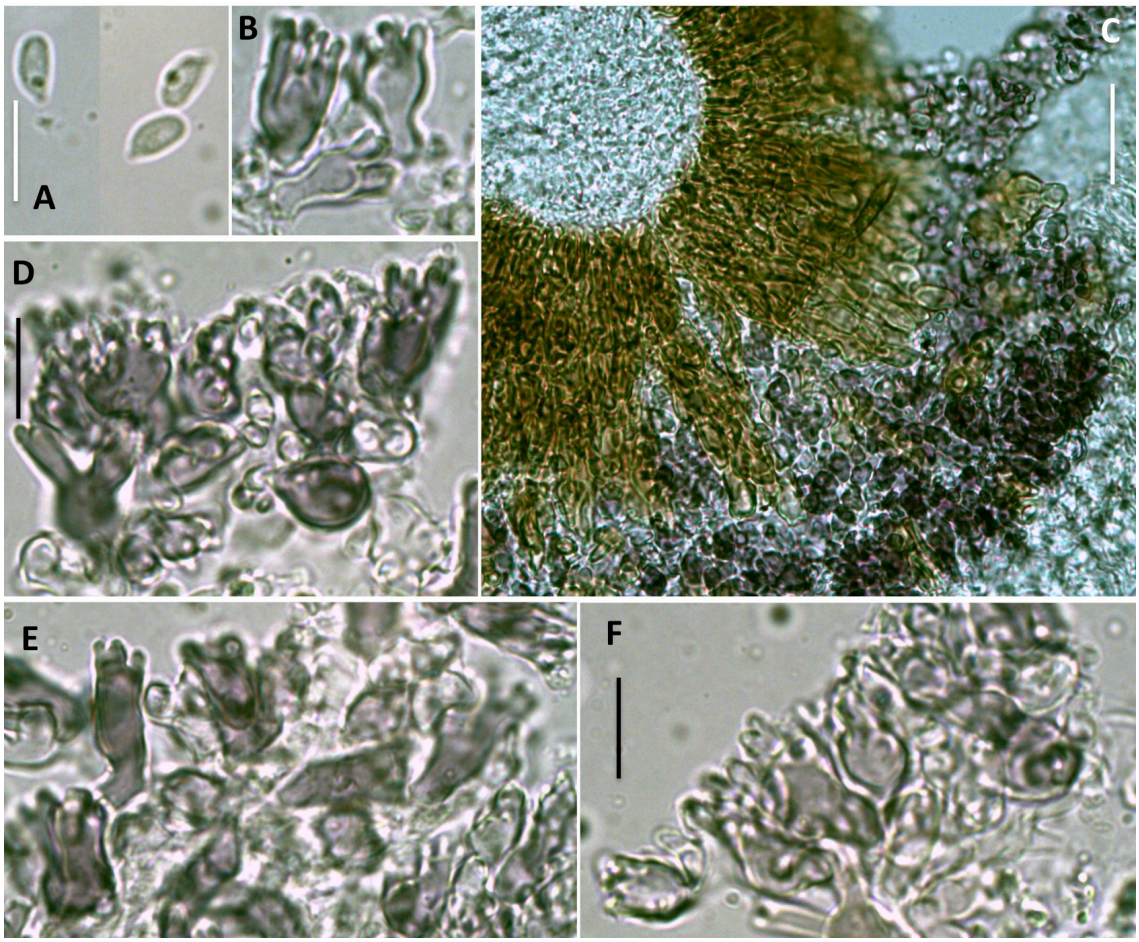


Fig.5A- *Marasmius populiformis*

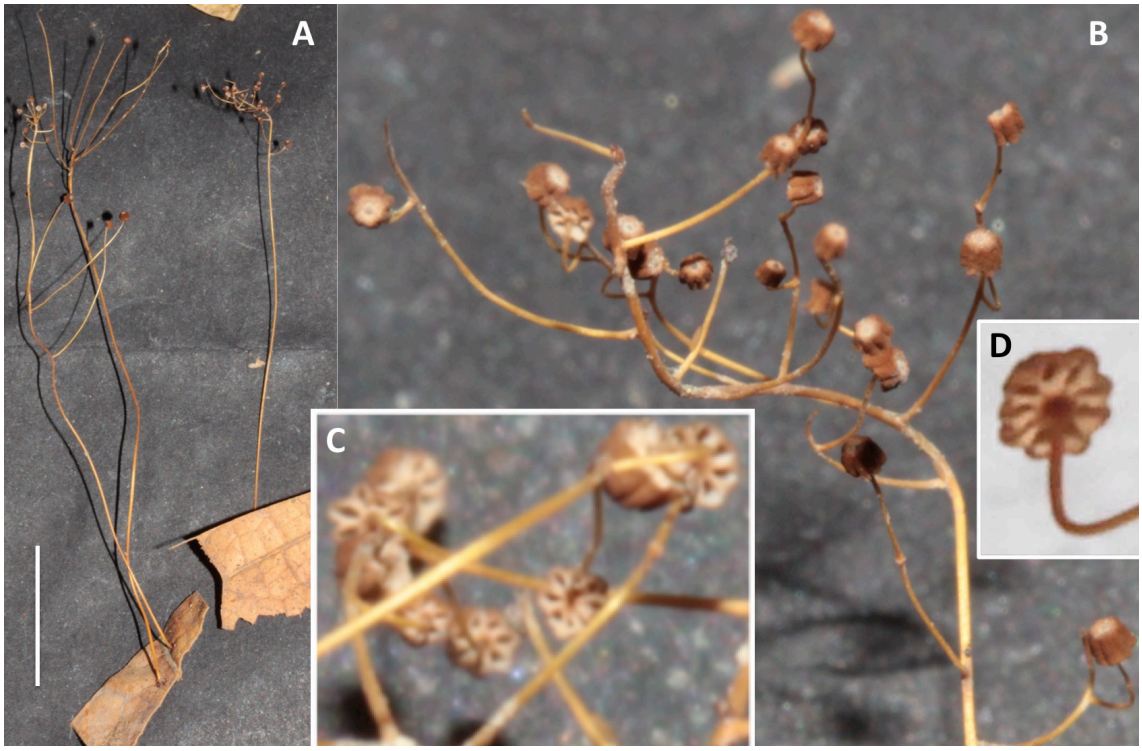


Fig.5B- *Marasmius populiformis*

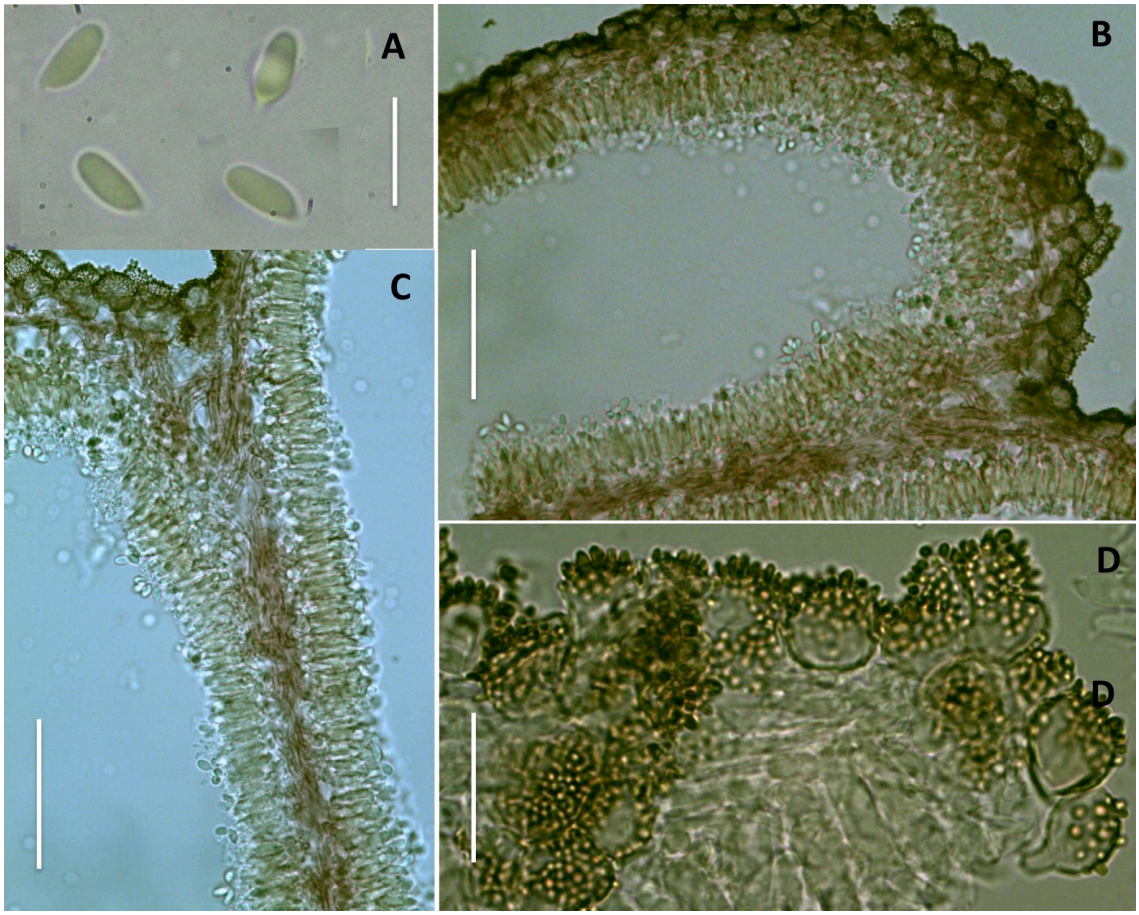


Fig.6A- *Marasmius* sp.1

Fig.6B- *Marasmius* sp.1

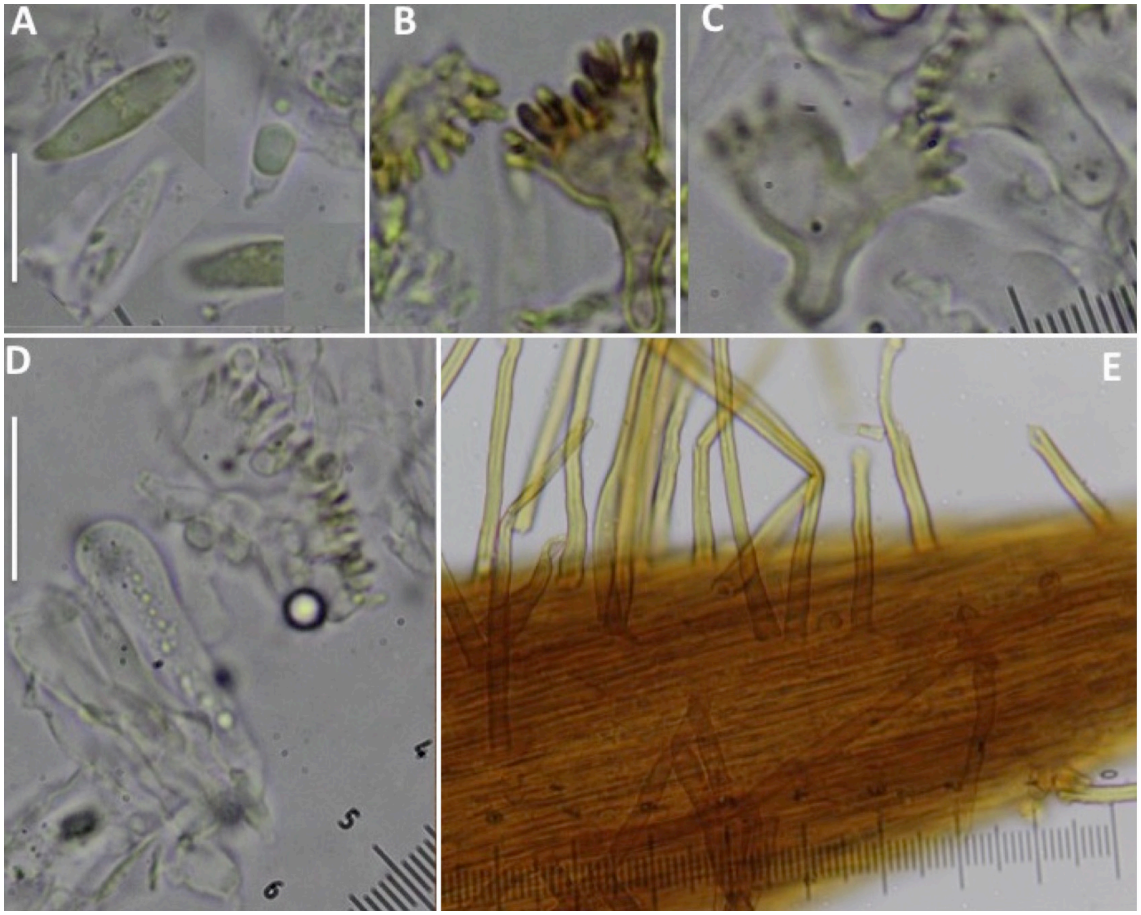


Fig.7A- *Marasmius* sp.2

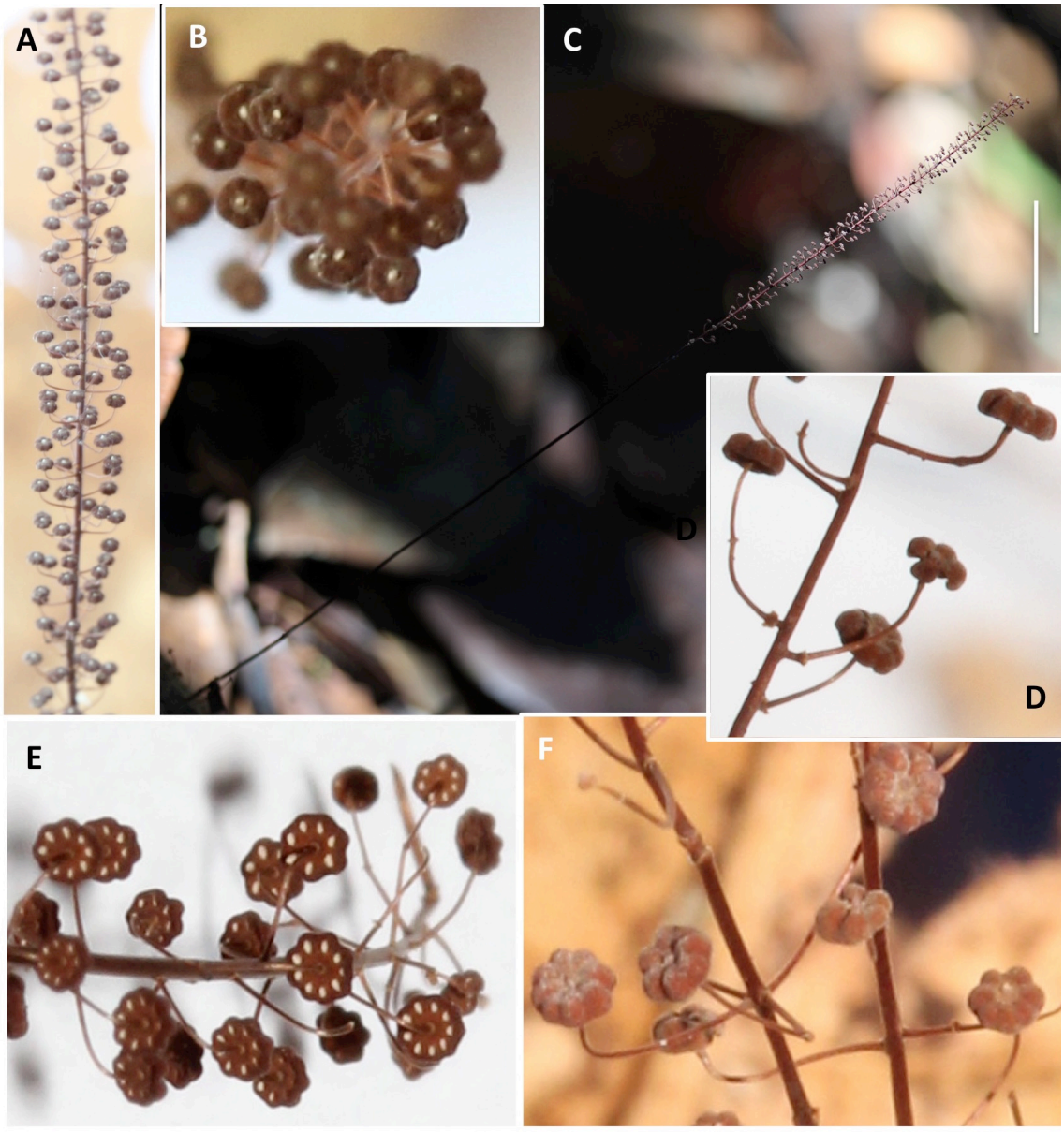


Fig.7B- *Marasmius* sp.2

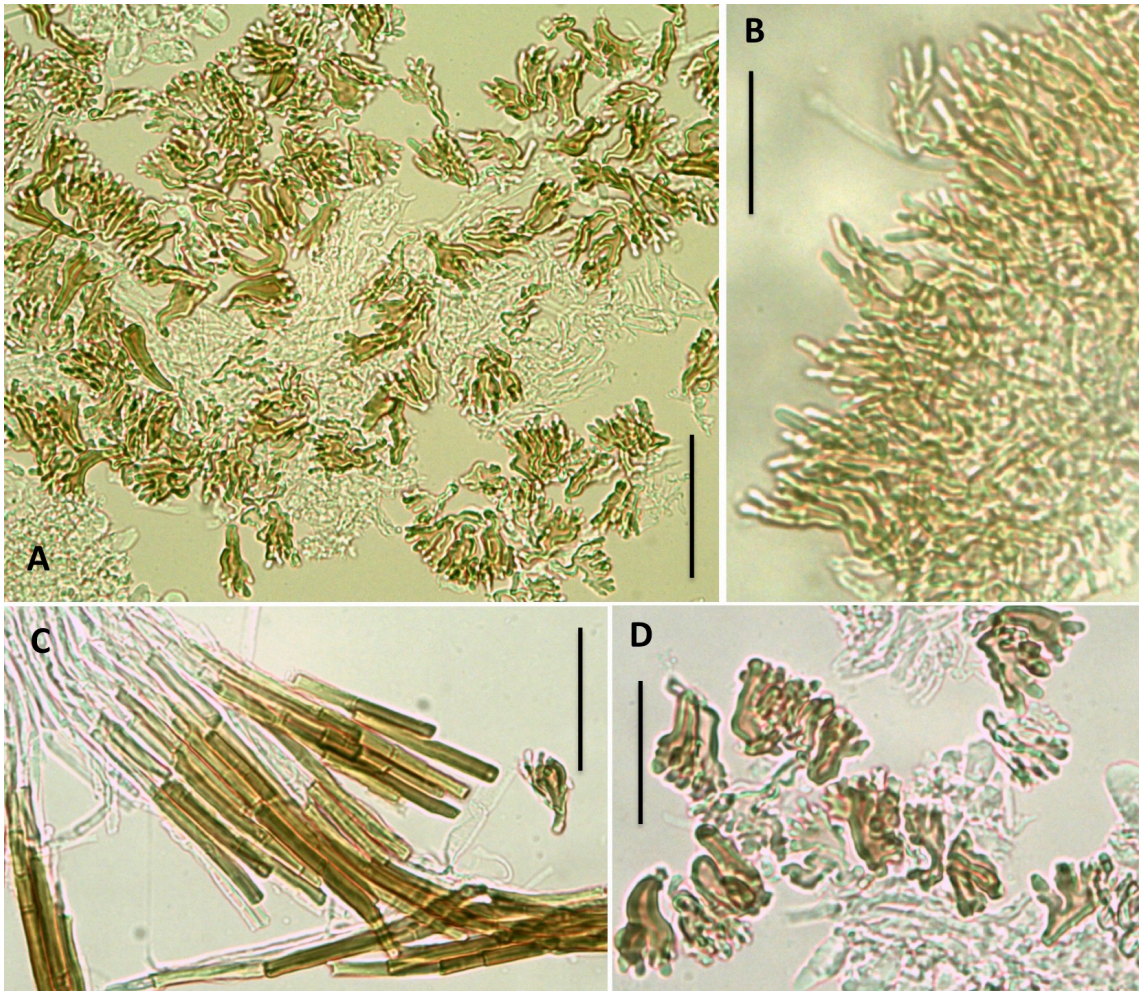


Fig.8A- *Marasmius* sp.3

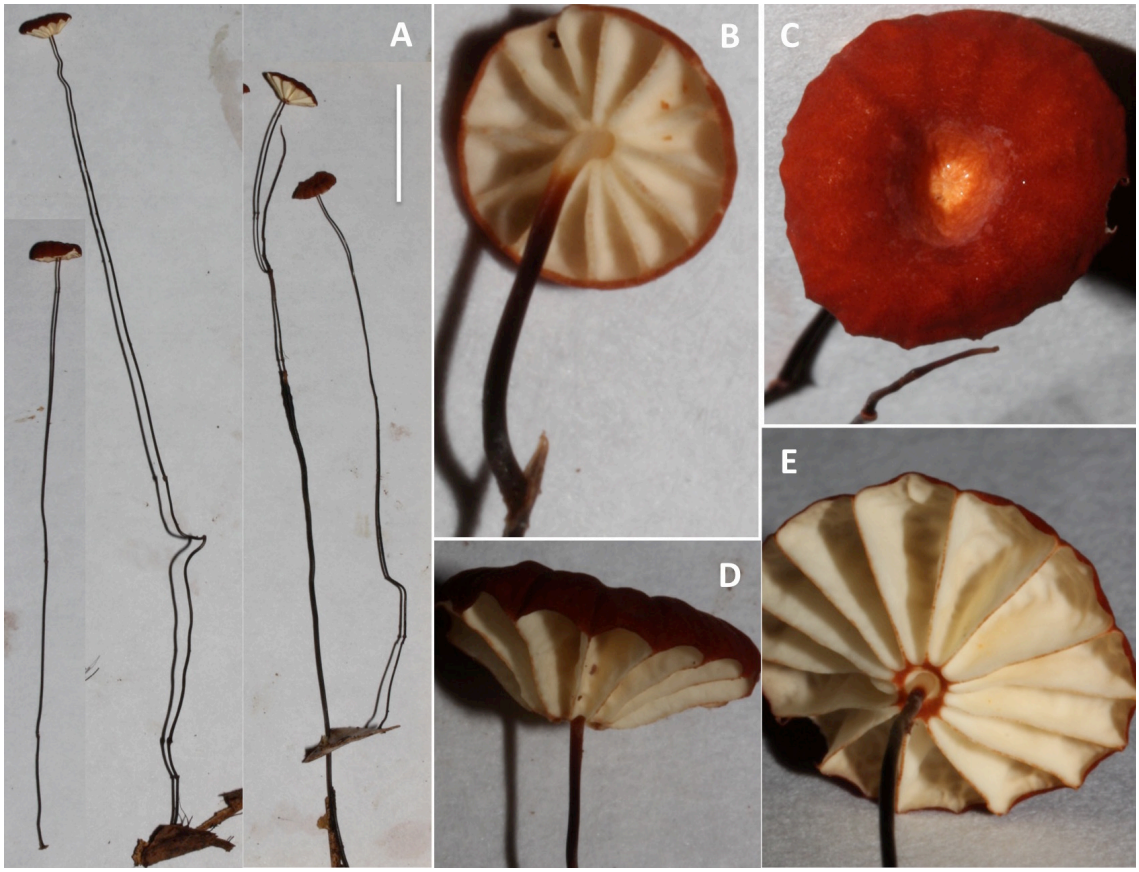


Fig.8B- *Marasmius* sp.3

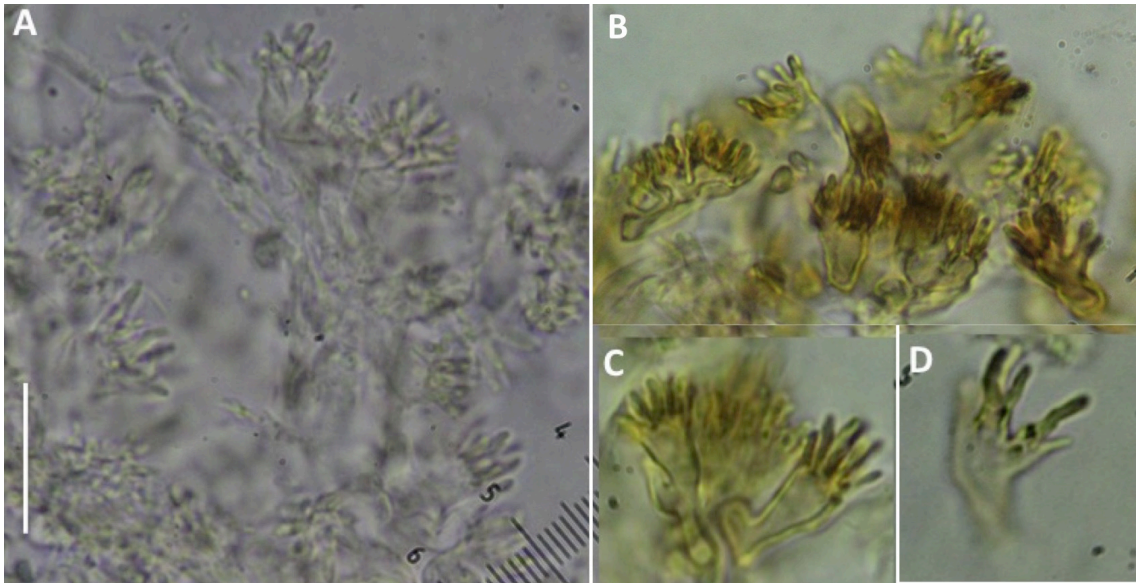


Fig.9A- *Marasmius* sp. 4

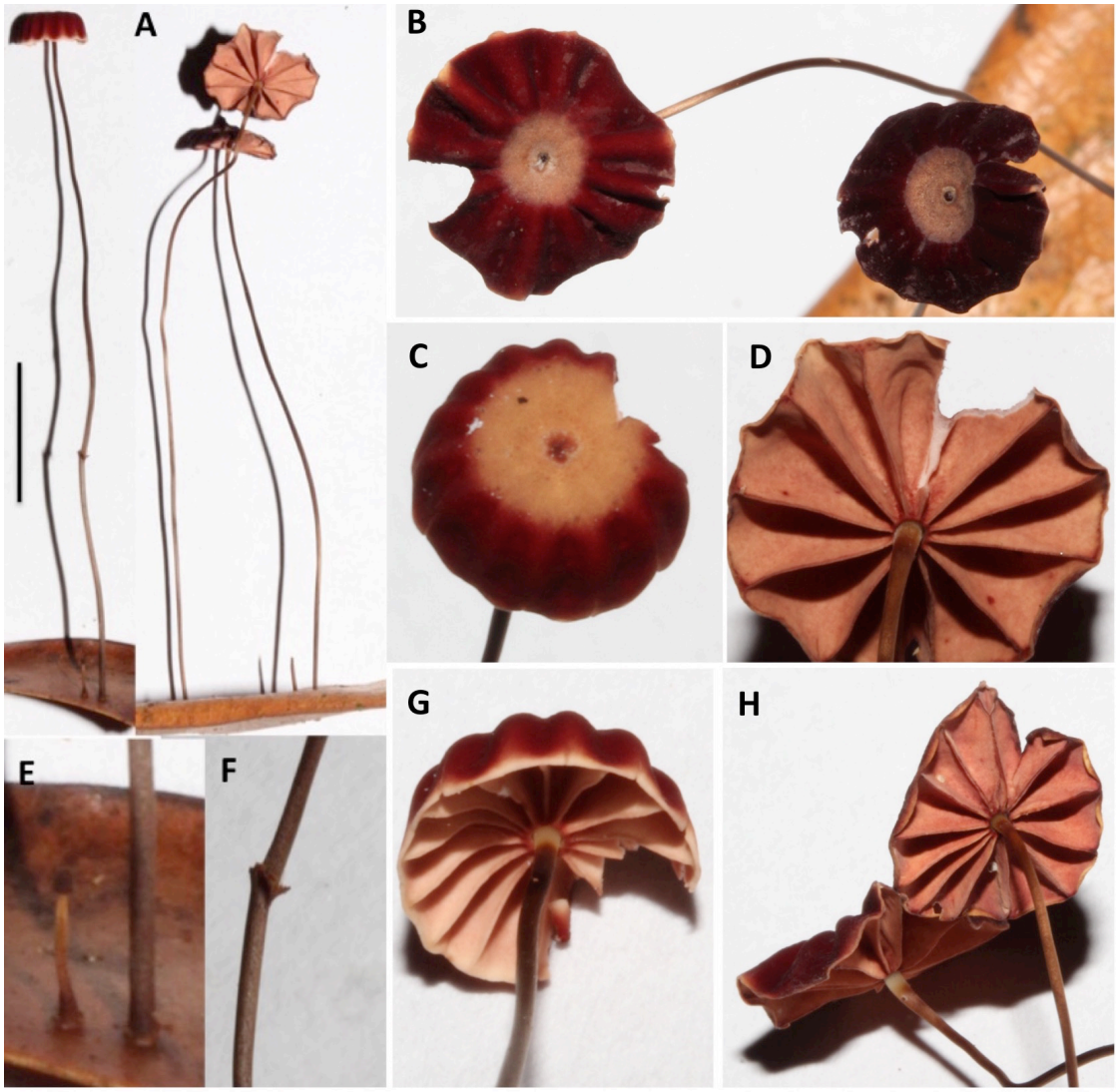


Fig.9B- *Marasmius* sp. 4

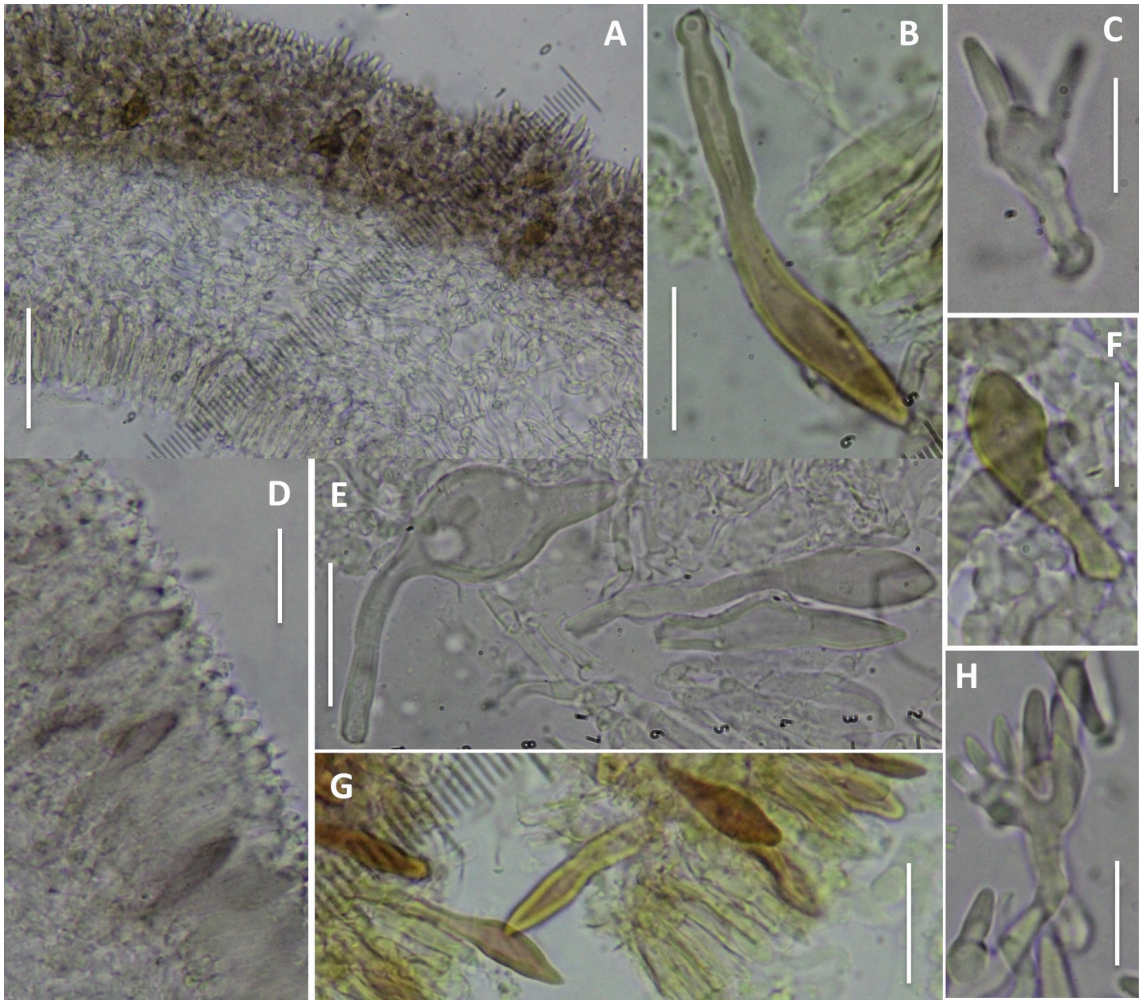


Fig.10A- *Marasmius* sp. 5

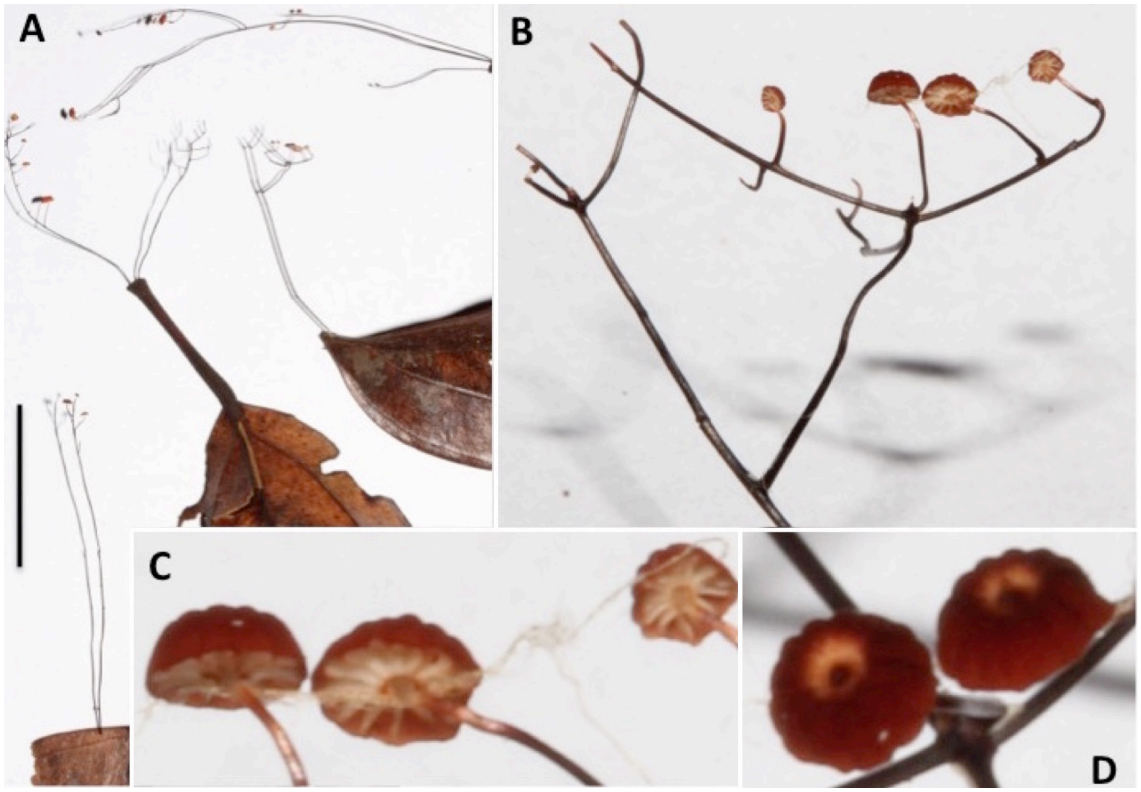
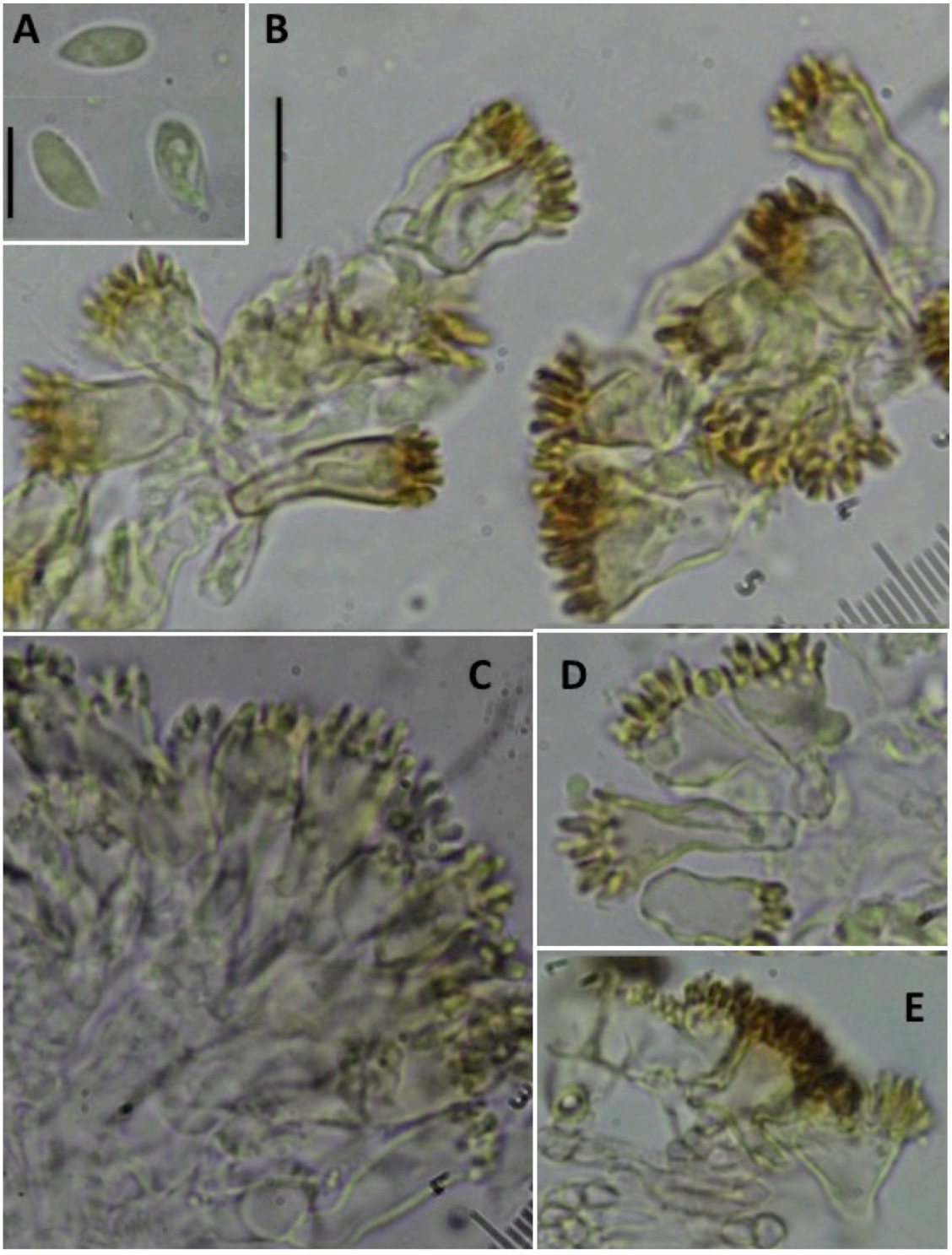


Fig.10B- *Marasmius* sp. 5



Capítulo 5

Komura, D.L; Oliveira, J. J. S.; Moncalvo, J. M.; Margaristescu, S.; Zartman, C. E. *Marasmius* from Amazonian terra firme forest of Brazil.

Manuscrito em preparação para *Fungal Diversity*

Marasmius spp. from Amazonian terra firme forest of Brazil

Dirce L. Komura[1*], Jadson J. S. Oliveira[2], Jean-Marc Moncalvo[2], Simona Margaritescu [2], Charles E. Zartman[1]

[1]Instituto Nacional de Pesquisas da Amazônia,Botany Department, C.P. 478, 69011-970 Manaus, Amazonas, Brazil

[2]Department of Natural History, Royal Ontario Museum & Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

Department of Natural History, Royal Ontario Museum, Toronto, Canada

National Institute for Amazonian Research, Department of Botany, C.P. 478, 69011-970 Manaus, Amazonas, Brazil

* E-mail address: leimybio@gmail.com

Abstract

Thirty-seven taxa of *Marasmius* from Amazonian terra firme forest in Brazil are described. ITS data, micro and macroscopic features are providing.

Key words: Amazonia – ITS – *Marasmius* – Neotropic – taxonomy

Introduction

Amazonia is the largest tropical forest in extension of the world, which is characterized by unique biomes, most formed by high and dense vegetation, but mosaics of white sand soil forest known as campina and campinarana (Prance 1975; Lisboa 1975) and floodplain areas as várzea and igapó (Junk and Piedade 2010). Here we presented *Marasmius* from terra firme forest, from platô where the forest never flood, is characterized by yellow latossol soil and vegetation very heterogeneous with diferent level of estrata (Jardim e Hosokawa 1986/87, Guillaumet 1987).

The genus *Marasmius* Fr. (Agaricales, Marasmiaceae) is worldwide distributed and about 500 species are described (Kirk et al. 2008) and 1,863 legitimate names are associated with this genus at MycoBank (http://www.mycobank.org), great of this diversity is concentrated in the tropics (Singer 1986), especially those from sections *Marasmius* (Rotulae) and *Sicci* are more diverse in South America than in Europe (Singer 1965). Representatives of this genus also was present in great number in ecological works, around 58 taxa (Braga-Neto 2007) and 78 taxa (Komura et al. 2016, umpublished) and was detected in brief period of collection. This shows that *Marasmius* spp. has a great role on the degradation of organic matter, especially in poor nutrient soil like Amazonian forest (Jordan 1982).

In relation of *Marasmius* taxonomy at Amazon, the works carried out by Rolf Singer was one of the most expressive at neotropics (Singer 1942, 1958, 1959, 1960, 1964, 1965, 1973, 1976, 1989; Singer and Digilio 1952). In 1976 he published the "Flora Neotropica" describing 233 spp. of *Marasmius*. At Mycobank, R. Singer has 314 legitimate names associated to this genus. Among the 60 species of *Marasmius* deposited at INPA herbarium, R. Singer has described 34 species.

The interpretation of the *Marasmius* taxonomy is a challenge, this is suggested in Singer (1953) comments "…what we call *Marasmius haematocephalus* is *Marasmius tageticolor* in Dennis, what we call *M. tageticolor* is an acystidiate species (the type) not mentioned in Dennis, and what Dennis calls *Marasmius haematocephalus* is a species unknow to me…", since that Singer (1986) organized the infrageneric level of *Marasmius* in 12 section, subsection and series, using characters as type of broom cells from epicutis, presence of pleurocystidia, amiloidity of the lamellar and pilear trama, etc.

Recently, based in molecular data, researchers observed that Singer classification not correspond a natural group (Owings and Desjardin 1997; Wilson and Desjardin 2005; Tan et al. 2009; Wannathes et al. 2009; Jenkinson et al. 2014; Oliveira et al. 2014). One of the first work about this issue, has used sequences of nuclear rDNA genes (25S, 5.8S, and ITS-1 and

2) from 17 species of *Marasmius* representing 7 different sections and the results have suggested that *Marasmius sensu stricto* were composed by the sects. *Marasmius*, *Sicci* and *Globulares* (Owings and Desjardin 1997). However, even within the *Marasmius sensu stricto*, the sections *Marasmius*, *Sicci* and *Globulares* did not form a monophyletic group (Tan et al. 2009; Wannathes et al. 2009; Oliveira 2014).

So based in morphological characters and r-DNA- ITS, this work presents descriptions of *Marasmius* from Amazonian terra firme forest.

Material and methods

Fieldwork: Specimens were collected during field expeditions in 2012-2015 at four sites in the Brazilian Amazon: (1) The Estação Experimental de Manejo Florestal do INPA (ZF-2) (02°37' S, 60°09' W), about 80 km north of Manaus, the state capital of Amazonas; (2) Instituto Nacional de Pesquisas da Amazônia campus (3° 05' 47" S, 59° 59' 14" W) in Manaus; (3) São Sebastião Community (2° 48' 04" S, 60° 29' 58" W), located at Cuieras river, a branched of Rio Negro river in Novo Airão and (4) Floresta Nacional do Tapajós (2° 51' 37" S, 54° 57' 57" W) at ICMBio Station Base Terra Rica, located at km 67 and at Comunidade Jamaraquá (2° 49' 45" S, 55° 01' 56" W), Belterra, Pará State.

Morphological descriptions: Freshly collected specimens were described macroscopically, and digital images were taken. We used the color code based on Color Picker chart color (http://www.colorpicker.com). Collections were dried at 40-50 °C with the use of an electric dehydrator (A. & J. Stöckli) or in silicagel for subsequent microscopical examination and herbarium preservation. The microscopic observation was carried out according to Oliveira and Capelari (2012). Sections of dried material were rehydrated in 70% ethanol and mounted in 5% KOH or in Melzer reagent for the amyloidity test. The dimensions of the spore measurements included the range of length × width, and following statistical analysis: X_m, the arithmetic Mean of length × width ± standard deviation; Q_m, the mean of the range of length/ width of basidiospores ± standard deviation; and n, the number of spores measured. The lamellae spacing was determined by the following factors: L, the number of lamellae that reach from the stipe to the pileus margin; and l, the number of series of lamellulae among the lamellae. The dried collections were deposited in the INPA and duplicates in the Royal Ontario Museum Fungarium (TRTC).

ITS sequences production and analysis: DNA isolation, PCR amplification, sequencing and editing of the ITS region followed Dentinger et al. (2010). Sequences showing >90% similarity to the newly produced *Marasmius* sequences were retrieved from BLAST (Altschul et al. 1990) searches in the NCBI database (GenBank), and aligned with our sequences using MUSCLE (Edgar 2004). A preliminary analysis was conducted in MEGA version 6 (Tamura et al. 2013) using Maximum-Likelihood (ML) and default parameters. From this preliminary analysis (data not shown) we selected 96 ITS sequences (Table S1) that were deemed to be relevant for this study. To analysis of the robusteness of the alignment sequences we used the web-based program Guidance (http://guidance.tau.ac.il/). ML settings for the final analysis were determined in jModeltest 2.1.7 (Darriba et al. 2012). The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model. The Bootstrap support (BS) for branches was estimated from 1,000 replications. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (2 categories (+G, parameter = 0.4963)). All positions with less than 90% site coverage were eliminated. That is, fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position.

Results and discussion

Phylogenetic analyses were carried out with 96 ITS sequences of *Marasmius*, with a total of 598 position in the final dataset. The reliable alignment of the ITS sequences dataset obtained in Guindance alignment score was 0.820817, this showed that most of the regions was accurately aligned. The tree with the highest log likelihood (- 6647.8517) is shown in Figure 1.

In general, the ITS was very usefull to species delimitation, specially those species with few distinctive morphological characters or collected in diferent stages of development. In addition, morphological taxonomy associated with molecular character can give a better understanding on the delimitation species of this genus. Although the ITS alone is limited to elucidation infrageneric structure, because all deep node of the tree presented with low support, some groups were very consistent, even in preliminary analysis (data not shown).

In the ML tree (Fig. 1), the clade "*M. haematocephalus*", contain seven sequences of *M. haematocephalus* from Thailand, Atlantic and Amazon forest from Brazil with high

support (98% BS). A similar result was found to *M. haematocephalus* complex to Thailand (Wannathes et al. 2009) and for specimens to Atlantic forest from Brazil (Oliveira 2014), for both works, the diferents variety and forms of *M. haematocephalus* formed a group with high support, suggesting a pantropical distribution and high conserved ITS sequences within the complex (Wannathes et al. 2009; Oliveira 2014).

The clade *Rotalis*-type, contain exclusively species collariate and pileipellis formed by *Rotalis*-type broom cells with 99% BS. This group belong to *Marasmius* subsection *Marasmius* is monophyletic; this also was corroborated in a work which used four species (Wannathes et al. 2009) and for specimens to Atlantic forest of Brazil (Oliveira 2014).

Species collariate, stipe insititious and pileipelis composed by *Siccus*-type broom cells have formed the clade B (70% BS), which is a sister group with moderated high support (81% BS) with clade *Rotalis*-type. However, the same morphological characteristics we can find in clade B and also clade A. The specimens in these two groups will be better analised to obtain any conclusion, about the divergences in these groups.

The great number of species in this study (except clade A, B and *Rotalis*-type) has stipe with mycelial pad and pileipelis composed by, at least, the *Siccus*-type broom cells. The presence of pleurocystidia is spread among this group and also the spore shape were not characters that can separate the groups.

Other relevants results about the ML tree are discussed in "Comments" for each taxon.

Taxonomy

1- *Marasmius* "orange8"

Figs. 2A and B

Pileus 15–20 mm diam, hemispherical to plane, striate, glabrous, opaque dark orange (8C2800) when young to pale orange (FFB682); *context* pale, thin. *Lamellae* adnate, irregular, some forked, crowded, *L*= 28, *l*= 1–2 series, narrow, smooth, whitish. *Stipe* 60–90 × 1 mm, central, cylindrical, hollow, wiry, polish, copper-brown turning cream to the apex; attached to substrate by mycelial pad.

Basidiospores 12.0 × 5.0 µm ellipsoid, smooth, hyaline, inamyloid. *Basidia* not observed. *Basidioles* most fusoid, some clavate. *Cheilocystidia* of *Siccus*-type broom cells, main body 15 × 6 µm, similar to pileocystidia but more hyaline and thin-walled. *Pleurocystidia* fusoid to ventricose-rostrate, some capitulate cells. *Pileipellis* hymeniform. *Pileocystidia* main body 15 × 6 µm, *Siccus*-type broom cells, some modified as thick, acute, long, setulae. *Pileus* and *Lamellar* trama dextrinoid.

Habit, habitat and known distribution: marasmioid, close, branch on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1201 (INPA259452); DLK1226 (INPA259479); 21 May 2013. D.L.Komura & O.F. Menezes, DLK1456 (INPA259454); D.L.Komura & F.C. Batista, DLK616 (INPA259450).

Comments: This taxon has morphological description similar to *M. spegazzinii* Singer (Singer 1976), but some microscopic features may have to check to confirm the identity, especially about the size of basidiospores and the presence of clavate, irregular, small bodies on the lamellar edge. The four sequences are grouped with 90% BS.

2- *Maramius* "orange23"

Figs. 3A and B

Pileus 4–10 mm diam, broadly convex, sulcate, glabrous, dull, dark-orange (CC3908) to red (A32900); *context* white, thin. *Lamellae* free, subdistant, *L*= 14, *l*= 2–3 series, smooth, white. Stipe 14–20 × 0.8 mm, central, cylindrical, copper-brown turning white to the apex; attached to substrate by white mycelial pad.

Basidiospores 12.0–13.0 × 4.0–4.5 µm [χ_m= 13.0±0.7 × 4.3±0.2 µm; Q_m= 3.0±0.3; n= 4], narrowly ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* not observed. *Basidioles* 23 × 6 µm, fusiform, thin-walled, hyaline. *Cheilocystidia* main body 10–15 × 5–9 µm, *Siccus*-type broom cells, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia* main-body 17–13 × 6–9 µm similar to cheilocystidia, composed of *Siccus*-type broom cells with brownish setulae. *Pileus trama* interwoven, dextrinoid, clamped. *Lamellar trama* parallel to interwoven, dextrinoid, clamped. *Stipitipellis* and *stipe trama* not observed.

Habit, habitat and known distribution: marasmioid, close, branches and leaves on the leaf litter at primary terra-firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1239 (INPA259488); 25 May 2013, D.L.Komura & F.C.Batista, DLK634 (INPA271938).

Comments: The two sequences of this taxon are clustered with high support (100% BS) in the phylogenetic tree, but we cannot infer any relationship among the *M.* "orange8" and *M. suthepensis*.

3- *Marasmius* cf. *suthepensis* Wannathes, Desjardin & Lumyong, Fungal Diversity 37: 288 (2009).

[MB#512429]

Holotype: CMU. **Type locality:** Thailand, Chiang Mai Province, Doi Suthep-Pui National Park, Mokfa Waterfall, on Hwy 1095.

Figs. 4A and B

Pileus 10–25 mm diam, campanulate to plan, slightly umbonate margin striate, glabrous, dull disc orange (F57C02) to margin pale orange (FCA349); *context* pale, thin. *Lamellae* free, subdistant, *L*= 16, *l*= 1–2 series, smooth, cream. *Stipe* 25–60 × 1 mm, central, cylindrical, hollow, wiry, polish, copper- brown turning cream to the apex; attached to substrate by mycelial pad.

Basidiospores 12.0–15.0 × 4.0–5.0 µm [χ_m= 13.1±1.0 × 4.9±0.3 µm; Q_m= 2.7±0.3; n=11], elipisoid to fusoid, thin-walled, hyaline, inamyloid. Basidia not observed. Basidioles 25.0 × 7.0 µm, clavate to fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia Siccus*-type, similar to pileocystidia, but hyaline. *Pleurocystidia* 35–40 × 4.0–6.0 µm, inconspicuous, clavate, capitulate, thin-walled, hyaline, *Pileipellis* hymeniform. *Pileocystidia*, main body 5.0–15.0 × 4.0–10.0 µm, setulae around 6.0 µm length. *Pileus trama* and *Lamellar trama* interwoven, strongly dextrinoid. Stipitipellis. Clamp connections present.

Habit, habitat marasmioid, close, leaf litter at secondary terra firme forest.

Known distribution: Congo, Kenya and Nigeria (as *M. ferruginoides*, Antonín 2004); Thailand (Wannathes et al. 2009); Brazil (Atlantic forest, São Paulo, Oliveira 2014).

Material examined: Brazil, Amazonas State, Manaus, Instituto Nacional de Pesquisas da Amazônia, 27 Feb. 2014, D.L.Komura, DLK1844 (INPA271966).

Comments: Our specimen is very similar to description for *M. suthepensis* from Thailand (Wannathes et al. 2009) and *M. ferruginoides* from Africa (Antonín 2004) and Brazil (Oliveira 2014). These names are synonims for the same species, according to Oliveira 2014. The sequences of *M. suthepensis* (JO329 and JO469) from Brazil are grouped in the same clade with moderate support (73% BS). The sequence from Thailand are collapsed and another sequence from Brazil (JO293) was placed in the clade with *M. "*orange8". However, Oliveira (2014) observed that the sequences of *M. suthepensis* from Brazil and Thailand have less than 1% of divergence.

4- *Marasmius* "orange24"

Fig. 5

Pileus 17 mm diam, broadly convex, center depressed sulcate, glabrous, dull red ferrugineous (702A13); *context* very thin. *Lamellae* free, with a rudimentar collar, subdistant, *L*= 14, *l*= 1 series, smooth, pale edge concolorous with pileus. *Stipe* 17 × 1 mm, central, cylindrical, dark brown turning pale to the apex; attached to substrate by cream mycelial pad.

Basidiospores not observed. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, inamyloid. *Cheilocystidia Siccus*-type, main body10 × 8 µm. *Pleurocystidia* absent. Pileipellis hymeniform. *Pileocystidia Siccus*-type main body 12 × 8 µm. *Pileus trama* and *Lamellar trama* dextrinoid. *Clamp connections* present in all tissues.

Habit, habitat and known distribution: marasmioid, solitary, branch on the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 22 May 2013, D.L.Komura & O.F.Menezes, DLK1483 (INPA259493).

5- *Marasmius* "orange1"

Figs. 6A and B

Pileus 5 mm diam, convex, umbonate slighty sulcate, rugulose, opaque, umbo red ferrugineous (962E0B) to light pink, brownish (FCD8B8); *context* pale, moderately thick. *Lamellae* adnate, subdistant, *L*= 13, *l*= 1 serie, smooth, edge brownish. *Stipe* 14–17 × 0.5 mm, central, cylindrical, dark brown turning pale to the apex; attached to substrate by cream mycelial pad.

Basidiospores 15.5–14.0 × 4.0–5.0 µm [χ_m= 15.5.0±0.3 × 4.6±1.0 µm; Q_m= 3.3±0.2; n=8] sub-fusoid to elongate clavate, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* clavate, fusoid, hyaline, thin-walled, inamyloid. *Cheilocystidia Siccus*-type, main body 12–10 × 4.0–6.0 µm. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia Siccus*-type, main body 12–10 × 4.0–6.0 µm, similar to cheilocystidia. *Pileus trama* and *Lamellar trama* dextrinoid. *Clamp connections* present in all tissues.

Habit, habitat and known distribution: marasmioid, close, leaf at leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May. 2012, D.L.Komura & O.F. Menezes, DLK499 (INPA259449).

6- *Marasmius* "brown2"

Fig. 7

Pileus 3–9 mm diam, convex to broadly convex, velutinous, dull, apaque, brown (40281B); *context* pale, thin. *Lamellae* free, subdistant, *L*= 13, *l*= one serie, edge smooth, brownish, concolorous with pileus. *Stipe* 5–8 × 0.5 mm, central, cylindrical, dark brown turning pale to the apex; attached to substrate by inconspicuous mycelial pad.

Basidiospores 16 × 5.0 µm, sub-fusoid to elongate clavate, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* main body 12 × 6.0 µm, *Siccus*-type. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. *Pileocystidia* main body 12 × 7.0 µm, *Siccus*-type similar to cheilocystidia. *Pileus trama* and *Lamellar trama* interwoven, dextrinoid. *Clamp connectios* present.

Habit, habitat and known distribution: marasmioid, close, leaf at leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK500 (INPA259631).

7- *Marasmius* "orange9"

Figs. 8A and B

Pileus 15–25 mm diam, campanulate, convex to plane-convex, center slightly depressed with umbo, sulcate margin some incurved or wavy, smooth, dull, opaque, orange (E88E5D) to dark orange (BF4E11); *context* pale very thin. *Lamellae* adnexed, subdistant, *L*= 12–13, *l*= 3–5, some very narrow, smooth, edge pale. *Stipe* 15–45 × 1–2 mm, central, cylindrical, polished, shiny, pale orange in young basidiomata turning orange brown in age, whitish to the apex; attached to substrate by cream mycelial pad.

Basidiospores not observed. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia Siccus* type similar to pileocystidia and modified *Siccus*-type as pleurocysitidia. *Pleurocystidia* main body 10–25 × 4.0–8.0 µm, composed by modified *Siccus*-type broom cells, thick-walled (2 µm), ochreaceous, with long, acute, thick-walled setulae. These cells cover all the lamellar side and the hymenium among lamellae. *Pileipellis* irregular to hymeniform. *Pileocystidia* main body 10–15 × 4.0–10.0 µm, *Siccus*-type broom cells. *Pileus trama* and *Lamellar trama* interwoven to parallel, dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, solitary to close, leaves on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 22 May 2012, D.L.Komura & F.C. Batista, DLK633 (INPA259457); Pará State, Belterra, Comunidade Jamaraquá, 25 Mar. 2014, D.L.Komura; T.S. Cabral & I.R. Fonseca, DLK1894 (INPA259460); ICMBio Base Terra Rica, km 67 da Rodovia , 28 Mar. 2014, D.L.Komura; T.S. Cabral & R. Oliveira, DLK1942 (INPA259461).

8- *Marasmius* cf. *griseoradiatus* Desjardin & Ovrebo, Fungal Diversity 21: 20 (2006). [MB#501122]

Holotype PMA. **Type locality:** Panama, Gatun Lake, Barro Colorado Island, Shannon Trail.

Figs. 9A and B

Pileus 16–20 mm diam, campanulate, sulcate, smooth, dull, opaque greyish brown (4D362A) with central disc and radial sulcate stripes white; *context* brownish, thin. *Lamellae* adnexed, distant, *L*= 9–10, *l*=0, smooth, brownish concolorous with pileus. *Stipe* 30–45 × 1mm, central, cylindrical, red brownish turning pale to the apex; attached to substrate by strigose, white mycelial pad.

*Basidiospores*12 × 5.0 µm fusoid to elongate clavate, thin-walled, hyaline, inamyloid. Basidia not observed. Basidioles clavate, thin-walled, hyaline, inamyloid. *Cheilocystidia* main body 8510 × 5.0–7.0 µm *Siccus*-type, similar to pileocystidia. *Pleurocystidia* main body 55 × 7.0 µm, elongate, capitulate, some bifurcate with obtuse apex, thin-walled, hyaline. *Pileipellis* irregular to hymeniform. *Pileocystidia* main body 10 × 7.0 µm, *Siccus*-type. *Pileus trama* and *Lamellar trama* interwove, to pararel, dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, clustery, leaf on the leaf litter at primary terra firme forest.

Material examined: Brazil, Pará State, Belterra, ICMBio Base Terra Rica, km 67, 28 Mar. 2014, D.L.Komura; T.S. Cabral & R. Oliveira, DLK1939 (INPA259737).

Comments: Description the species from Panama (Desjardin and Ovebro 2006) is similar to our specimen. This species was described to Mato Grosso, Brazil (Lodge and Sourell 2015).

9- *Marasmius* aff. *phaeus*

Figs. 10A and B

Pileus 12–20 mm diam, campanulate, broadly campanulate to convex, sulcate, velutinous, dull, shiny pale orange (FA803E) with central disc yellowish, and radial stripes whitish to dark orange (D44106); *context* white, thick. *Lamellae* adnexed, distant, *L*= 7–9, *l*= 0, smooth, opaque, edge concolous with pileus. *Stipe* 15–25 × 1 mm, central, cylindrical, polished, red brownish turning pale to the apex, attached to substrate by strigose, pale mycelial pad.

Basidiospores not observed. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* main body 10–12 × 4.0–8.0 µm, *Siccus*-type. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. *Pileocystidia* 10–12 × 4.0– 6.0 µm main body *Siccus*-type, similar to cheilocystidia. *Pileus trama* and *Lamellar trama* interwoven, dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, clustery, leaf on the leaf litter at primary terra firme forest.

Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, 25 Mar. 2014, D.L.Komura; I.R. Fonseca & T.S. Cabral, DLK1895 (INPA259376).

Comments: Our specimen is similar to *Marasmius griseoradiatus* described to Panama (Desjardin and Ovebro 2006), differing in the pileus color, on which the specimen from Panama is grey and our, is orange. This species is related to *M. tageticolor*, but presents pleurocystidia. Also is related to *M. phaeus* description, but the color is diferent, not dark reddish brown, although the lamellae edge are concolous with the pileus as our specimen. *Marasmius montagneanus* Singer (Singer 1976) has pileus shape and color similar, but difer in the lamellae features and also the presence of pleurocystidia. But more inspections to find basidiospores is necessary to complete description of our material to define the species.

10- *Marasmius* "orange5"

Figs. 11A and B

Pileus 4–6 mm diam, convex to broadly convex, sulcate papilla in young basidiomata flat, turning darker and conspicuous in age, subvelutinous, opaque central disc orange (D6681E), pale orange to brownish (DEB18C) to turning red (5E220A) with lighter stripes; *context* cream, thin. *Lamellae* adnate, distant, *L*= 7–9, *l*= 0, smooth, opaque edge concolorous with the pileus, more evident in young basidiomata. *Stipe* 14–20 × 0.6 mm, central, wiry, opaque, brownish turning pale to the apex, attached to substrate by strigose, pale mycelial pad.

Basidiospores 13 × 4.0 µm, ellipsoid to lacrimoid, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* 15–18.0 × 5.0-–6.0 µm clavate, fusoid, thin-walled, hyaline, inamiloid. *Cheilocystidia Siccus* type, which was present on the lateral of the lamellae continuos, similar to pileocystidia. *Pleurocystidia* 25–30.0 × 6.0 µm elongate, some capitulate, mucronate, clavate, afillate. Pileipellis hymeniform. *Pileocystidia* around 8.0 × 6.0 µm, *Siccus*-type. *Pileus trama* and *Lamellar trama* interwoven, dextrinoid. *Stipitipellis* parallel, thick-walled, dextrinoid cells. *Caulocystidia* short, cylincric cell, bastonete-like forms, hyaline, inamyloid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf on the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Apr. 2012, D.L.Komura & P.A.Pereira, DLK1359 (INPA259492); 24 May 2012, D.L.Komura & O.F.Menezes, DLK1580 (INPA271957).

11- *Marasmius haediniformis* Singer, Bull. Jard. Bot. Bruxelles 34: 363. 1964. [MB#333705]

Holotype BR. **Type locality:** Zaire, Des Lacs Edouard et Kivu, Pare National Albert, environs d'Hoysha (from Mycobank typification [MB#163485]); Congo, Leopoldville on the protologue).

Figs. 12A and B

Pileus 14–19 mm diam, plan to wavy, sulcate revolute, smooth, dull, opaque pure white; *context* white, thin. *Lamellae* adnate, distant, *L*= 10, *l*= 2 series, white, lamellulae forked. *Stipe* 10–16 × 2 mm, central, cylindrical, turning slightly clavate to the apex, velutinous at the base, attached to substrate by white, short, strigose mycelial pad, wine-brownish turning white to the apex.

Basidiospores 13 × 4 µm, fusoid to clavate, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia Siccus*-type, thin-walled, hyaline. *Pleurocystidia*, as cystidiole, clavate, thin-walled, inamyloid, inconspicuous, scarcely projecting from hymenial layer, neither deeply attached to lamellar trama. *Pileipellis* hymeniform, inamyloid. *Pileocystidia Siccus*-type, thin walled, hyaline, inamyloid. *Pileus context* interwoven hyphae and *lamellar trama* irregular, strongly dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf on the leaf litter at primary terra firme forest. Equador, Zaire and Brasil (Atlantic forest, São Paulo).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK502 (INPA271934).

Comments: This species at first, is macroscopically similar to *M. niveus* (Singer 1976) according to description of the pileus shape and stipe, presence of basal mycelium, but microscopically is completely distint with pileocystidia smooth and clavate-pedicellate, while our specimen has *Siccus*-type broom cells. Also differs from *M. haedinus* in having well differentiated *Siccus*-type broom cells and numerous pleurocystidia unlike our specimen.

12- ***Marasmius congregatus*** Mont., Annales des Sciences Naturelles Botanique Serie 1: 113 (1854).

[MB#215237]

Holotype K (?). **Type locality:** French Guyana (?).

Figs. 13A and B

Pileus 15–45 mm diam, plane, margin striate, revolute, hygrophanous cream (FCECCA); *context* pale, thin. *Lamellae* adnate, crowded, *L*= >25, *l*= 3, smooth, very narrow, concolorous, slighty intervenose. *Stipe* 20–40 × 3 mm, central, cylindrical, hollow, polished, red brownish turning pale to the apex, pale, strigose mycelial pad.

Basidiospores 8.0 × 3.0 µm, lacrimoid, thin-walled, hyaline, inamyloid. *Basidia* clavate, 4-sterigmate, thin-walled, hyaline, inamyloid. *Basidioles* around 20.0 × 5.0 µm, clavate, some fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia Globulares* and *Siccus*-type, similar to pileocystidia, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* hymeniform to irregular. *Pileocystidia* composed by 2 types of broom cells: 1)- *Globulares*-type 20.0 × 15.0 µm main body, thin-walled, hyaline; 2)- *Siccus*-type 12.0 × 8.0 µm main body, thin-walled, hyaline. This last one interspersed among the *Globulares*-type. *Pileus trama* and *Lamellar trama* interwoven dextrinoid, dimitic: 1) thinner, up to 5 µm, opaque; 2) around 8.0 µm, inflated and hyaline hyphae. *Stipitipellis* parallel, thick-walled, cylindrical hyphae. *Caulocystidia* versiform, clavate, fusoid, irregular, thick-walled. *Clamp connections* present.

Habit, habitat: gymnopoid, caespitose, branches on the leaf litter at primary secondary forest.

Known distribution: Pantropical, Indonesia, New Caledonia, Singapore, Thailand (Wannathes et al. 2004), Brazil (Puccinelli and Capelari 2009; Oliveira 2014).

Material examined: Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, 24 Mar. 2014, D.L.Komura; I.R. Fonseca & T.S. Cabral, DLK1866 (INPA271970).

Comments: Our specimen is very similar one described from São Paulo (Oliveira 2014) and also for redescription of *Marasmius congregatus* from holotype collected in French Guiana (Wannathes et al. 2004). These authors also report the descriptions of *Marasmius pellucidus* from South Asia, a species macroscopically identical to our specimen, but belong to section *Globulares*. Oliveira (2014) describes *Amyloflagelulla*-type caulocystidia in *M. congregatus* from São Paulo we found just an irregular form as versiform, clavate, fusoid caulocystidia. The ITS sequences of the both samples were placed in the same group with high support (100% BS) in the preliminary phylogenetic tree.

13- *Marasmius* "orange10"

Fig. 14

Pileus 5–12 mm diam, convex, ferruginous to orange brown (9E5B21); *context* pale, thin. *Lamellae* adnate, distant, *L*= 9, *l*= 2, some forked, pale, concolorous, smooth. *Stipe* 7–25 × 1 mm, cylindrical, smooth, brown to white upward. Long strigose, white, mycelial pad.

Basidiospores 10 × 4.0 µm fusoid to clavate, thin-walled, hyaline, inamyloid. Basidia not observed. *Basidioles* clavate, slighty fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* few number, *Siccus*-type, similar to pileocystidia. *Pleurocystidia* Pileipellis irregular. *Pileocystidia* main body 8–10 × 4.0–7.0 µm, *Siccus*-type, similar to cheilocystidia. *Pileus trama* and *Lamellar trama* irregular to paralles, dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf on leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1299 (INPA 259464)

14- *Marasmius bellus* Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 139 (1856).

[MB#209092]

Holotype ?. **Type locality:** Brazil, Amazonas, Panuré.

Figs. 15A and B

Pileus 10 mm diam, convex, margin sulcate, smooth pure white; *context* white, thin. *Lamellae* adnate, subdistant, *L*= 13, *l*= 2, pure white, intervenose, forked, reticulate, appearing poroid. *Stipe* 48 × 2 mm, central, cylindrical, opaque, attached to substrate by white, strigose mycelial pad, red brownish turning white to the apex.

Basidiospores 10 × 3.0 µm fusoid, thin-walled, hyaline, inamiloid. Basidia not observed. *Basidioles* fusoid and clavate, thin-walled, hyaline, inamyloid. *Cheilocystidia* just in few numbers present, *Siccus*-type. *Pleurocystidia* inconspicuous, maybe cystidioles, fusoid, capitulate, thin-walled, hyaline, inamyloid. *Pileipellis* hymeniform. *Pileocystidia* main body 10–14 × 5–7 µm, *Siccus*-type, similar to cheilocystidia. *Pileus trama* and *Lamellar trama* dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, solitary, leaf on the leaf litter at primary terra firme forest. Brazil and Bolivia (Singe 1976).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 Sep. 2013, D.L.Komura & J.R. Maciel, DLK1736 (INPA 259505).

Comments: Our specimen is similar to macroscopic characteristics from Berkeley protologue (1856) and from Singer (1965, 1976) both macro and microscopical features. In the ITS analysis our sequence are placed with *M. bellus* from Atlantic Forest of Brazil (JO299) with high support (96% BS). The macroscopic description has slightly differences about the pileus color (more cream and yellow in JO299) and the lamellae, our specimen is clearly intervenose, maybe our basidiome is an immature stage and this character are changing with age, turning less poroid.

15- *Marasmius* "orange2"

Figs. 16A and B

Pileus 35- 20 mm diam, convex to plan, striate, dull, opaque orange pale (FFA75E) to orange (C9460E); *context* orange, thin. *Lamellae* free, subdistant, *L*= 13, *l*= 2, smooth, narrow pure white, intervenose, some forked, more evident in young basidiomata. *Stipe* 20–50 × 1–2 mm, central, cylindrical, hollow, opaque, red brownish turning white to the apex; attached to substrate by white, strigose mycelial pad; superficial micelia present on the susbtrate.

Basidiospores 9.0 × 4.0 µm, ellipsoid, smooth, hyaline, inamyloid, thin-walled. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia Siccus*-type broom-cells. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia* composed by *Siccus*-type and modified *Siccus*-type with long, thick-walled setulae. *Pileus trama* and *Lamellar trama* regular, parallel, dextrinoid. *Stipitipellis* paralled, thick-walled cell, dextrinoid. *Caulocystidia Amyloflagelulla*-type broom cells, dextrinoid. *Clamp connections* present .

Habit, habitat and known distribution: marasmioid, solitary, branch on the leaf litter at primary and secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 May 2012, D.L.Komura & F.C.Batista, DLK676 (INPA 259435); 24 Aug. 2012, D.L.Komura, DLK892 (INPA271941); Instituto Nacional de Pesquisas da Amazônia, 27 Feb. 2014, D.L.Komura & L.S. Bento, DLK1843 (INPA271965).

16- *Marasmius ruber* Singer, Sydowia 18: 263, 342 (1965).

[MB#240643]

Holotype: LIL. **Type locality:** Bolivia, El Beni, Vaca Diez, Guayaramerin.

Figs. 17A and B

Pileus 10–30 mm diam, convex to broadly convex, margin striate, smooth, dull, shiny in young basidiomata red orangish (FA3A00) to orange (FA7D16); *context* orange, thin.

Lamellae adnate, subdistant, *L*= 10–12, *l*= 3 series, edge concolorous with according the pileus. *Stipe* 11–32 × 1mm, central, cylindrical, polished, yellowish cream to flesh color all cream when quite young; attached to substrate by white, strigose mycelial pad.

Basidiospores 10 × 4.0 µm, fusoid, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamlyloid. *Cheilocystidia* present in few numbers of *Siccus*-type broom cells. *Pleurocystidia* absent. *Pileipellis* irregularly hymeniform. *Pileocystidia Siccus*-type broom cells. *Pileus trama* and *Lamellar trama* interwoven, weakly dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, decayed branch at primary terra firme forest. Brazil, Bolivia, Trinidad (Singer 1976).

Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, 24 Mar. 2014, D.L.Komura, T.S. Cabral & I. R. Fonseca, DLK1864 (INPA259745). Comunidade Maguari, 30 Mar. 2014, D.L.Komura, T.S. Cabral & O. Dias, DLK1966 (INPAXXX).

Comments: Our specimen is similar to Singer description (1976). Its ITS sequences is similar to DED8669 from Atlantic forest of Brazil with high support (94% BS).

17- *Marasmius* "orange4"

Figs. 18A and B

Pileus 10–18 mm diam, convex, umbonate, plan, slightly sulcate, margin striate, dull orange reddish (A13800) to orange (F28950); *context* pale orange to white, thin. *Lamellae* adnexed to free, subdistant, *L*= 12–14, *l*= 3 series, edge discolorous with pileus, white to pale, forked. *Stipe* 20–45 × 1 mm, central, cylindrical, opaque, velutinous at the base, attached to substrate by pale, strigose mycelial pad, wine-brownish turning white to the apex.

Basidiospores 8.1–11.6 × 3.1–4.0 µm [χ_m= 9.9±1.1 × 3.5±0.3 µm; Q_m= 2.8±0.3; n=11], narrowly ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* 20 × 6, clavate, 4-sterigmate, thin-walled, hyaline. *Basidioles* 17 × 7, fusoid, thin-walled, hyaline. *Cheilocystidia* main body 10–12 × 5–7 µm, *Siccus*-type broom cells, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. Pileocystidia 10–8 × 4–9 µm main body, *Siccus*-type broom cells similar to cheilocystidia, but brownish setulae. *Pileus trama* interwoven, dextrinoid. *Lamellar trama* parallel to interwoven, dextrinoid. *Stipitipellis* and *Stipe trama* parallel, thick-walled. *Caulocystidia* absent.

Habit, habitat and known distribution: marasmioid, close, leaf ont the leaf litter at secondary and primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1356 (INPA271949); 10 Sep. 2013, D.L.Komura & J.R. Maciel, DLK1725 (INPA 259738).

Comments: This taxon is close related to *M. cladophyllus* with high support in the phylogenetic tree (99% BS).

18- ***Marasmius cladophyllus*** Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 138 (1856).

[MB#240643]

Holotype: ?. **Type locality:** Brazil, Amazonas, Panuré.

Figs. 19A and B

Pileus 11–23 mm diam, broadly convex to plan, smooth, dull, opaque, orange (F76A1E); *context* cream to orangish, thin. *Lamellae* adnate, close, *L*= 14–16, *l*= 3 series, margin some concolorous with the lamellae, some concolorous with the pileus, white to orange-cream, intervenose, forked, appearing poroid. *Stipe* 15–40 × 1 mm, central, cylindrical, opaque, subvelutinous, brown turning white to the apex, completely white when young; attached to substrate by pale, strigose mycelial pad; superficial micelia present on the susbtrate.

Basidiospores 10 × 4 μm, fusoid to clavate, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* present in few number of *Siccus*-type broom cells. *Pleurocystidia* absent. Pileipellis irregular to hymeniform. *Pileocystidia* 12 × 7 μm main body, *Siccus*-type. *Pileus trama* and *Lamellar trama* interwoven, dextrinoid. *Stipitipellis* parallel, thick-walled cells. *Caulocystidia* absent. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf on the leaf litter at primary and secondary terra firme forest. Argentina, Belize, Bolivia, Brazil, Ecuador, USA (Singer 1976).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 18 Apr. 2012, D.L.Komura, J.M. Moncalvo, C.E. Zartman, DLK289 (INPA 271928); 10 May 2012, D.L.Komura & T.S. Marinho, DLK506 (INPA271935); 23 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1192 (INPA271945); 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1256 (INPA 259739); 22 May 2013, D.L.Komura & O.F.Menezes, DLK1464 (INPA 259740);

Comments: This specimen is according to Singer (1976), it is possible that is *Marasmius cladophyllus* var *cladophyllus*.

19- *Marasmius* cf. *digilioi* Singer, Singer & Digilio, Lilloa 25: 201. 1952.
[MB#300180]

Holotype: LIL. **Type locality:** Argentina, Tucumán, Sierra de San Javier.

Figs. 20A and B

Pileus 23 mm diam, broadly convex, margim striate, opaque, dull, velutinous, brownish-olivaceous (85704E); *context* cream, thin. *Lamellae* adnexed, close, *L*= 24, *l*= 1–2 series, concolorous with the pileus. *Stipe* 30 ×1 mm, central, cylindrical, bulbous at apex, polished, wine-brownish turning cream to the apex, attached to substrate by pale, strigose mycelial pad.
Basidiospores 10 × 4 µm, ellipsoid, thin-walled, hyaline, inamyloid. *Basidia* not observed. *Basidioles* fusoid, thin-walled. *Cheilocystidia Siccus*-type, setulae thick-walled. *Pleurocystidia* absent. *Pileipellis* irregular ro hymeniform. *Pileocystidia Siccus*-type broom cells. *Pileus trama* and *Lamellar trama* parallel to interwoven, strongly dextrinoid. Stipitipellis parallel, cylindrical, thick-walled, dextrinoid cells. *Caulocystidia* somewhat *Siccus*-type and modified in long, thick-walled setulae. *Clamp connections* preent.
Habit, habitat: marasmioid, solitary, leaf litter at secondary terra firme forest.
Known distribution: Bolívia, Argentina (Singer 1976).
Material examined: Brazil, Amazonas State, Manaus, Instituto Nacional de Pesquisas da Amazônia, Campus I, 31 Jan. 2014, D.L.Komura & L.S. Bento, DLK1826 (INPA271961).
Comments: This species is according to Singer (1976) descriptions and is related to "olivaceous" clade, with *M. trinitatis* with moderate support (84%BS).

20 *Marasmius* cf. *trinitatis* Dennis, Transactions of the British Mycological Society 34 (4): 425 (1951).
[MB#300230]

Holotype: ?. **Type locality:** Trinidad and Tobago, Trinidad, Sangre Grande.

Figs. 21A and B

Pileus 25 mm diam, broadly convex with margin plan, sulcate striate, smooth, dull, opaque, brown (694000); *context* greyish-cream, thin. *Lamellae* adnexed, subdistante, *L*= 14, *l*= 3–4 series, cream. *Stipe* 20 × 2 mm, central, cylindrical, opaque, pruinose, attached to substrate by pale, strigose mycelial pad, brown turning cream to the apex.
Basidiospores 11 × 4 µm, fusiform to elipsoid, thin-walled, hyaline, inamyloid. *Basidia* clavate, 4-sterigmate. *Basidioles* fusoid and clavate. *Cheilocystidia Siccus*-type. *Pleurocystidia* absent. *Pileipellis* irregular ro hymeniform. *Pileocystidia Siccus*-type, similar to cheilocystidia. *Pileus trama* and *Lamellar trama* parallel to irregular, *Stipitipellis* parallel,

cylindrical, thick-walled, dextrinoid cells. *Caulocystidia* modified *Siccus*-type broom cells with long, thick-walled setulae. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, solitary, decay branch at terra firme forest.

Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, 25 Mar. 2014, D.L.Komura; T.S. Cabral & I.R. Fonseca, DLK1896 (INPA271971).

Comments: This specimen is very similar to Dennis (1951) description for type from Trinidad, except for the number of lamellae (here less numerous). Recent description from São Paulo (Oliveira 2014) about the caulocystidia showed similar with our sample. This is one of the olivaceous *Marasmius*, other species that occur in Amazon are *M. digilioi* and *M. epelaeus* (Singer 1965, 1976).

21- *Marasmius phaeus* Berk. & M.A. Curtis, Botanical Journal of the Linnean Society 10: 298 (1869).

[MB#210676]

Holotype: ?. **Type locality:** Cuba.

Fig. 22A and B

Pileus 10- 22 mm diam, campanulate, umbonate sulcate, opaque, velutinous ferrugineous (873305); *context* white, thin. *Lamellae* adnate, but attached to a reduced collar, distant, *L*= 9–10, *l*= 0, the center of hymenium between lamellae concolorous with the pileus, while pileus margin and lamellae white edge concolorous with pileus, some white as lamellae. *Stipe* 30–40 × 1 mm, central, cylindrical, wiry, smooth, polished, dark-brown turning pale on the apex, attached to substrate by pale, strigose mycelial pad.

Basidiospores 17.8–20.8 × 3.5–4.5 µm [χ_m= 19.5±0.9 × 4.0±0.3 µm; Q_m= 4.9±0.4; n=30], narrowly clavate to cylindrical ellipsoid, smooth, hyaline, inamyloid, thin-walled. *Basidia* 30 × 7 µm, clavate, 4-sterigmate, thin-walled, hyaline with drops. Basidioles 25–27 × 5.5–6.5, clavate to cylindrical, thin-walled. *Cheilocystidia Siccus*-type broom cells, brownish setulae. *Pleurocystidia* absent. Pileipellis irregular to hymeniform. *Pileocystidia Siccus*-type broom-cells, some thick-walled, brownish setulae. *Pileus trama* interwoven, dextrinoid. *Lamellar trama* irregular to parallel, dextrinoid. *Stipitipellis* and *stipe trama* not observed.

Habit, habitat and known distribution: marasmioid, clustery, decay trunk at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 19 Apr 2012, D.L.Komura, J.M. Moncalvo & C.E. Zartman, DLK326 (INPA271929); 7 May 2012, D.L.Komura & T.S. Marinho, DLK412 (INPA271931); 10 May

2012, D.L.Komura & T.S. Marinho, DLK485 (INPA271933); 22 Aug. 2012, D.L.Komura, DLK882 (INPA 259375); 22 May 2013, D.L.Komura & O.F.Menezes, DLK1472 (INPA271954).

Comments: Our specimen is similar to description in Singer (1976).

22- ***Marasmius hypophaeus*** Berk. & M.A. Curtis, Botanical Journal of the Linnean Society 10: 298 (1869).

[MB#195008]

Holotype: ?. **Type locality:** Cuba.

Figs. 23A and B

Pileus 5–30 mm diam, onvex to brodly convex, sulcate slightly umbilicate, opaque, dull, rugulose purple-redish (70122D) in young basidiome, orange ferrugineous (9E482E) with purple-reddish disc; *context* white to orangish. *Lamellae* free to adnexed, seem to attached to a reduced collar, subdistant, *L*= 17, *l*= 0, white edge orange ferrugineous. *Stipe* 10–30 × 0.8 mm, central, cylindrical, wiry, tortuous, smooth, polished, dark-brown turning purplish to rose on the apex, in immature basidiomata, purple-reddish; attached to substrate by white, narrow, strigose mycelial pad, on whitish superficial mycelia convering the substrate.

Basidiospores 15.0–18.0 × 4.0–5.0 µm [χ_m= 16.4± 1.0 × 4.7± 0.5 µm; Q_m= 3.5± 0.4; n=15], narrowly clavate to cylindrical ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* clavate, 4-sterimate, thin-walled, inamyloid. *Basidioles* clavate, thin-walled, inamyloid. *Cheilocystidia Siccus*-type. *Pleurocystidia* inconspicuous among hymenium, clavate, acuminado, capitulate. *Pileipellis* irregular to hymeniform. *Pileocystidia Siccus*-type. *Pileus trama* and *Lamellar trama* interwoven, dextrinoid. *Clamp connections* present.

Habit, habitat: hematocephaloid, gregarious, decay trunk at terra firme forest.

Known distribution: Cuba, Colombia, Ecuador, Mexico (Singer 1976), Indonesia (Desjardin et al. 2000), Brazil (Puccinelli & Capelari 2000, Oliveira 2014).

Material examined: Brazil, Amazonas State, Manaus, INPA- Campus I, 14 Feb. 2014; D.L. Komura; M.R. Pereira & P.A. Pereira, DLK1833 (INPA271963).

Comments: Due its high variation in color, size described by many author, our specimen is characterized by description in Singer (1976) and Oliveira (2014), hematocephaloid habit, hymantium-like pellicula on the substrate and ferrugineous color in some degree according to development stage of the basidiomes, stipe tortuous and dark-brown. However, unlike it these descriptions our specimen showed inconspicuous pleurocystidia, which were observed after removed from the hymenial layer. The shape is similar from those descriptions cited above. In relation to ITS analysis, our specimen is placed in the same clade with *M. hypophaeus* from

Atlantic forest of Brazil (JO454, JO387, JO438), presenting moderate support (84% BS). However, within this clade, our sequence seems to be divergente, keeping isolated from sequences from Atlantic forest (98% BS).

23- ***Marasmius haematocephalus*** (Mont.) Fr., Epicrisis Systematis Mycologici: 382 (1838). [MB#244588]

Holotype: not extant. **Type locality:** Brazil, Rio de Janeiro.

Figs. 24A and B

Pileus 7–12 mm diam, convex to campanulate, sulcate, opaque, subvelutinous red-wine (521215) to red (E63538); *context* white to reddish. *Lamellae* adnate, distant, *L*= 8, *l*= 0, mebranaceous, white; a white mebranaceous layer cover along the hymenium between lamellae in young specimens. *Stipe* 20–45 × 0.8 mm, central, cylindrical, wiry, smooth, polished, dark-brown turning pale on the apex, attached to substrate by white, pruinose mycelial pad.

Basidiospores not observed. *Basidia* not observed. *Basidioles* clavate, fusiform, thin-walled, hyaline, inamyloid. *Cheilocystidia* scantteded, *Siccus*-type. *Pleurocystidia* conspicuous, abundant, 40–50 × 6–10 µm, fusoid, some with acute apex, thin-walled, hyaline, inamyloid. *Pileipellis* hymeniform. *Pileocystidia Siccus*-type, main body 8–15 × 5–10 µm, thin-walled, setulae thicker and ochraceous. *Pileus trama* and *Lamellar trama* strongly dextrinoid. *Clamp connections* present.

Habit, habitat: hematocephaloid, scattered to gregarious on leaf litter at primary terra firme forest.

Known distribution: Pantropical, South and Central America (Singer 1965, Oliveira 2014), Thailand (Wannathes et al. 2009), Malaysia (Tan el al. 2009), Africa (Pegler 1966), Oceania (Desjardin & Horak 1997).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 May 2013, D.L.Komura & O.F.Menezes, DLK1579 (INPA271956).

Comments: This species has very variable pileus shape (applanate to campanulate) and color (orange to dark purple). Singer (1976) has described at least nine varieties. Recently, Wannathes et al. (2009) described five forms to Thailand and Oliveira (2014), four forms to Atlantic forest from Brazil. This species is macroscopically similar to *Marasmius tageticolor*, but *M. hematocephalus* present conspicuous pleurocystidia.

24- *Marasmius berteroi* (Lév.) Murrill, North American Flora 9 (4): 267 (1915).
[MB#357314]

Holotype: ?. **Type locality:** Puerto Rico.

Figs. 25A and B

Pileus 18 mm diam, convex applanate, slightly sulcate, subvelutinous, orange (D65311) with red (7D1D0E) disc on the center; *context* orangish-cream, thin. *Lamellae* free, distant, *L*= 9, *l*= 1 series, edge concolorous with the pileus, some not. *Stipe* 28 × 2 mm, central, cylindrical, smooth, translucid, wine-red turning water-green to the apex, attached to substrate by pale, strigose mycelial pad; superficial micelia present on the susbtrate.

Basidiospores 15–20 × 3.5–5.0 µm [χ_m= 17.0 ±1.5 × 4.1±0.4 µm; Q_m= 4.2±0.3; n=10], oblong to slightly clavate, thin-walled, hyaline, inamyloid. *Basidia* clavate, 4-sterigmate, thin-walled, hyaline. *Basidioles* clavate and fusoid. *Cheilocystidia Siccus*-type, hyaline, not numerous. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia Siccus*-type with thick-walled, ochraceous setulae. *Pileus trama* and *Lamellar trama* parallel to irregular, dextrinoid. *Clamp connections* present.

Habit, habitat: marasmioid, solitary, leaf litter at terra firme forest.

Known distribution: Bristish Honduras, Bolivia, Argentina, Brazil.

Material examined: Brazil, Amazonas State, Manaus, Instituto Nacional de Pesquisas da Amazônia, 12 Mar. 2014, D.L.Komura & P.A.Pereira, DLK1849 (INPA271967);

Comments: This species is close related *M. rhabarbarinoides* in the ITS analysis with high support (99%).

25- *Marasmius* "orange7"

Fig. 26

Pileus 4–6 mm diam, convex aplanate, sulcate umbilicate, opaque, dull, subvelutinous orange to red (8A2C0C); *context* pale, thin. *Lamellae* adnate, or attached to a reduced collar, distant, *L*= 10–12, *l*= 0, cream-brownish. *Stipe* 10–16 × 0.5 mm, central, cylindrical, wiry, smooth, polished, brownish-copper, apex cream; insititious to substrate.

Basidiospores not observed. *Basidia* not observed. *Basidioles* fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* not observed. *Pleurocystidia* absent. *Pileipellis* hymeniform to irregular. *Pileocystidia* somewhat transition of *Siccus*-type and *Rotalis*-type, some with main-body globular with short setulae and other with thick-walled, setulae shorter than traditional *Siccus*-type broom cells. *Pileus trama* and *Lamellar trama* inamylid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, gregarious, branch on the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1276 (INPA 259474).

Comments: This specimen is morphological similar to *Marasmius* "orange3", but their molecular sequence are completly distint.

26- *Marasmius* cf. *guyanensis* Mont., Annales des Sciences Naturelles Botanique 1: 114 (1854).

[MB#244345]

Holotype: ?. **Type locality:** French Guiana.

Figs. 27A and B

Pileus 2–5 mm diam, campanulate, sulcate umbilicate with black papila, opaque, dull, rugulose yellow-orange ocher (FFC16B), dry material orange (E88A07), umbilicus darker and margin lighter; *context* pale, thin. *Lamellae* attached to collar, distant, *L*= 10, *l*= 0, cream. *Stipe* 10–22 × 0.3 mm, central, cylindrical, filiform, black to dark-brown, insititious; telepods present.

Basidiospores 11 × 5 μm, ellipsoid, hyaline, inamyloid. *Basidia* not observed. *Basidioles* clavate and fusoid, thin-walled, inamyloid. *Cheilocystidia Siccus*-type. *Pleurocystidia* absent. *Pileipellis* hymeniform *Pileocystidia Siccus*-type, main body short (5–10 μm), some thick-walled, ochraceous. *Pileus trama* regular, very narrow, weakly dextrinoid. *Lamellar trama* regular, weakly dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Instituto Nacional de Pesquisas da Amazônia, Campus I, 27 Feb. 2014, D.L.Komura M.R. Pereira & P.A.Pereira, DLK1832 (INPA271962); DLK1837 (INPA271964).

Comments: This species is according to description in Singer (1976), but in the ITS phylogeny, are completely distint when compared with the sequences from Atlantic forest. This taxon need more attention to understand this results.

27- ***Marasmius tageticolor*** Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 136 (1856)

[MB#157809]

Holotype: ?. **Type locality:** Brazil, Amazonas, Panuré.

Figs. 28A and B

Pileus 3–26 mm diam, convex to campanulate, sulcate, pale pink (D69C9E) to magenta (B02848); *context* pinkish to cream, very thin. *Lamellae* adnate to adnexed, distant, *L*= 9–10, *l*= 0–3, white to cream. *Stipe* 11–33 × 1 mm, central, wiry, smooth, polished, dark-brown turning water-green to the apex, in imature basidioma, major water-green; attached to substrate by pale, narrow, strigose mycelial pad.

Basidiospores 17.6–21.9 × 3.3–4.4 µm [χ_m= 19.8±1.1 × 3.8±0.3 µm; Q_m= 5.2±0.5; n=26], narrowly clavate to cylindrical ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* 26–31 × 5.5–8.5 µm, clavate, 2-,4-sterigmate, thin-walled, hyaline. *Basidioles* 25–28 × 5.8–7 µm, clavate, fusoid, thin-walled, hyaline. *Cheilocystidia* main body 10–15 × 4–10 µm, *Siccus*-type broom cells, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform, brownish. *Pileocystidia* main body 10–17 × 5–13 µm, similar to cheilocystidia, brownish setulae. *Pileus trama* interwoven, dextrinoid, clamped. *Lamellar trama* interwoven to parallel, dextrinoid, clamped. *Stipitipellis* regular without any differentiated structures. *Stipe trama* parallel cells, thick-walled. *Caulocystidia* absent.

Habit, habitat: marasmioid, solitary to gregarious, on leaf litter at terra firme forest. *Known distribution:* Brazil, Trinidad, Dominica, Bahamas (Dennis 1951, Singer 1976), Malaysia (Desjardin et al. 2000), Mexico, Venezuela (Singer 1976).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK508 (INPA271936); 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1369 (INPA271950); 26 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1399 (INPA271951); 11 Set 2013, D.L.Komura & J.R. Maciel, DLK1746 (INPA271958).

Comments: Our specimen is very similar to specimen described by Berkely (1856) to Amazonas and Singer (1976), but differ in the pileus color, originaly have ocher radial stripe and the stipe base with abundant, pure white basal mycelium. This morphophology also was described to Panamá (Desjardin and Ovebro 2006). However, a watercolor painting commissioned by van Overeem, a Dutch mycologist who worked out of Herbarium Bogoriense in Java in the early part of the last century, is identical to our material and was identified as *M. tageticolor*. The description of *M. tucumanus* (Singer 1976) in Argentina also is similar to our specimen, but the pileus size (6–11 mm) and the spore length (11.5–13.7 ×

3.5–4 μm) is smaller. This species differ from *M. haematocephalus* described here for the absence of pleurocistidia.

In relation to ITS data, this taxon showed slightly differences in the sequences, but its sequences are grouped with moderate support (88% BS) and are close related to *M.* cf. *lilacinoalbus* with high support (100% BS).

28- *Marasmius* cf. *lilacinoalbus* Beeli, Bulletin de la Société Royale de Botanique de Belgique 60 (2): 158 (1928).
[MB#269465]
Holotype: ?. **Type locality:** Congo, Central Africa.

Figs. 29A and B

Pileus 10–40 mm diam, convex, campanulate and broadly parabolic, sulcate, dull, slightly shiny, peach color (EBAB78) with red-wine (8C0E29) radial stripes; *context* white, thin. *Lamellae* adnexed, distant, *L*= 12, *l*= 0, opaque white. *Stipe* 75–100 × 1 mm, central, wiry, smooth, polished, insititious, dark-brown to copper-brown overall.
Basidiospores 20–23 × 4.5–6.0 μm [χ_m= 21.2±1.1 × 5.0 ±0.4 μm; Q_m= 4.3 ±0.4; n=15], narrowly clavate to cylindrical ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* clavate, 4-sterigmate, thin-walled, inamyloid. *Basidioles* clavate, similar to basidia. *Cheilocystidia Siccus*-type, scant. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia Siccus*-type. *Pileus trama* and *Lamellar trama* interwove, weakly dextrinoid. *Clamp connections* present.
Habit, habitat and known distribution: hemtocephaloid, close, leaf litter at terra firme forest.
Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, ICMBio Base Terra Rica, km 67, 28 Mar. 2014, D.L.Komura; T.S. Cabral & R. Oliveira, DLK1932 (INPA 259383).
Comments: The strikingly striped pileus and color is similar to *M. lilacinoalbus* Beeli from Africa (Singer 1964). Just the stipe diam, is thinner (up to 1 mm), while african species reaches 2 mm diam. Microscopics features are similar in both.

29- *Marasmius leoninus* Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 135 (1856).

[MB#198914]

Holotype: K(M). **Type locality:** Brazil, Amazonas.

Figs. 30A and B

Pileus 10–35 mm diam, convex to plan, striate, dull, opaque, smooth to rugose pale orange (FFBD73); *context* cream to pale, thin. *Lamellae* adnexed to free, distant, *L*= 14–18, *l*= 3–6 series, cream to pale orange. *Stipe* 10–20 × 1mm, central, cylindrical, wiry, smooth, polished, yellow ferrugineous, apex cream and thicker, attached to substrate by white, narrow, white, shortly strigose mycelial pad.

Basidiospores 13.0–16.0 × 4.5- 5.0 µm [χ_m= 14.4±1.2 × 4.9±0.2 µm; Q_m= 2.9±0.3; n=10], oblong to ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* clavate, 4-sterigmate, thin-walled, hyaline. *Basidioles* clavate, thin-walled, hyaline. *Cheilocystidia* main body 8-12 × 5-10 µm, *Siccus*-type, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* regular, brownish broom cells. *Pileocystidia* main body 5–17 × 7–15 µm, similar to cheilocystidia *Siccus*-type, but with browinish setulae. *Pileus trama* interwoven, dextrinoid, inflate cells around 8 µm diam, brownish. *Lamellar trama* interwoven, thin-walled cells. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, clustery, branch on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 21 May 2012, D.L.Komura & F.C. Batista, DLK591 (INPA271937); 23 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1215 (INPA259478); 26 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1414 (INPA 259382).

30- *Marasmius* "orange21"

Figs. 31A and B

Pileus 7–30 mm diam, convex to plan, sulcate incurved, some revolute, dull, opaque, smooth to rugose pale orange (F29D3D) to orange (A34100). *Context* cream to pale, thin. *Lamellae* adnexed to free, distant, *L*= 11- 13, *l*= 0- 1 series, cream to pale orange. *Stipe* 1–2 × 15–30 mm, central, cylindrical, wiry, smooth, polished, dark-brown to copper-brown overall, pale-salmon in young basidiomes attached to substrate by white, narrow, strigose mycelial pad.

Basidiospores 11.6–16.0 × 3.1–4.5 µm [χ_m= 14.2±1.5 × 3.8±0.4 µm; Q_m= 3.8±0.4; n=13], narrowly clavate to cylindrical ellipsoid, smooth, hyaline, thin-walled inamyloid. *Basidia* clavate, thin-walled, hyaline. *Basidioles* clavate, thin-walled, hyaline. Cheilocystidia main

body 10–17.3 × 5–7.3 µm, *Siccus*-type, thin-walled, hyaline. *Pleurocystidia* absent. *Pileipellis* regular, brownish broom cells. *Pileocystidia* main body10–17.3 × 5–7.3 µm, similar to cheilocystidia *Siccus*-type, but with browinish setulae. *Pileus trama* interwoven, dextrinoid, inflate cells around 8 µm diam, brownish, clamped. Lamellar trama interwoven to parallel, hyphae 4 µm diam, thin-walled clamped. *Stipitipellis* and *stipe trama* not observed.

Habit, habitat and known distribution: marasmioid, clustery, branch on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 May 2012, D.L.Komura & F.C.Batista, DLK690 (INPA271939); 23 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1217 (INPA271946); DLK1241 (INPA 259489); 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1249 (INPA271947); 26 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1413 (INPA271953).

Comments: This species is similar to description in Dennis (1951a) and Oliveira (2014). All the three sequences are clustered in the phylogenetic tree with high support (98% BS).

31- *Marasmius* cf. *jalapensis* Murrill, North American Flora 9: 264. 1915.
[MB#200160]

Holotype: NY. **Type locality:** Mexico, near Jalapa.

Figs. 32A and B

Pileus 45 mm diam, broadly convex, center depressed, sulcate, hygrophanous, cream-brownish (BA885B); *context* pale, thin. *Lamellae* adnate, subdistante, *L*= 16, *l*= 1–2 series, pale, narrow. *Stipe* 80 × 3mm, central, cylindrical, bulbosous at apex, opaque, velutinous to pruinose, attached to substrate by pale, short, strigose mycelial pad, red-brownish along the stipe, superficial micelia present on the susbtrate.

Basidiospores not observed. *Basidia* not observed. *Basidioles* not observed. *Cheilocystidia* and *Pleurocystidia* very conspicuous and abundant setae, present on the side and edge of the lamellae, 40–100 × 6–12 µm, lanceolate, apex acute, thick-walled (wall 2–3 µm of thick), ochreaceous. *Pileipellis* irregular to hymeniform. *Pileocystidia Siccus*-type, some thick-walled and ochreaceous, setae scanted. *Pileus trama* and *Lamellar trama* interwoven, strongly dextrinoid. *Stipitipellis* parallel, cylindrical, thick-walled, dextrinoid. *Caulocystidia* somewhat *Siccus*-type broom cells with setulae long and thick-walled, setae similar from the lamellar. *Clamp connections* present.

Habit, habitat: gymnopoid, solitary, leaf litter at terra firme forest.

Known distribution: Bolivia, Mexico, Venezuela (Singer 1965, 1976).

Material examined: Brazil, Pará State, Belterra, Comunidade Jamaraquá, 25 ICMBio Base Terra Rica, km 67, 28 Mar. 2014, D.L.Komura; T.S. Cabral & R. Oliveira, DLK1944 (INPA271973).

Comments: Our specimen corresponds to Singer (1976) description, but an analysis from the spores is necessary to confirm the identification. At first view, this species belong to sect. *Sicci* subsect *Siccini* ser *Actinopodus* due setoid elements present on pileus or stipe or lamellae, or else on several of these surfaces; cystidia present or absent on the lamellae (Singer 1976). This species is close to *M. atrorubens* but without support.

32- *Marasmius* "orange3"

Fig. 33

Pileus 2–5 mm diam, convex to broadly convex, sulcate, umbilicate, opaque, dull, orange (822D03); *context* pale, thin. *Lamellae* attached to collar, distant, *L*= 11, *l*= 0, cream. *Stipe* 10–15 × 0.3 mm, central, cylindrical, filiform, smooth, polished, dark-brown, apex pale, insititious.

Basidiospores 9.0–11.0 × 4.5–5.0 µm [χ_m= 10.3±0.7 × 4.9± 0.3 µm; Q_m= 2.1±0.2; n=7], ellipsoid to lacrimoid, thin-walled, hyline, inamyloid. *Basidia* not observed. *Basidioles* fusoid, clavate, thin-walled, hyaline, inamyloid. *Cheilocystidia Siccus* type, similar to pileocystidia. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia* two type of *Siccus*-type 1) main body 8.0–15.0 × 7.0–15.0 µm, thin-walled, setulae ocher; 2) main body 15.0–20.0 × 7.0–10.0 µm, thin-walled. *Pileus trama* and *Lamellar trama* interwoven, inamyloid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, clustery, leaf petiole on the leaf litter at terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1338 (INPA259438).

33- *Marasmius* "orange16"

Fig. 34

Pileus 4–9 mm diam, broadly convex aplanate, sulcate, umbilicate with brown papila, opaque, dull, velutinous orange (C4561F); *context* pale, thin. *Lamellae* attached to collar, distant, *L*= 10, *l*= 0, white edge concolorous with pileus. *Stipe* 0.5 × 15–20 mm, central, cylindrical, wiry, smooth, polished, dark-brown, apex whitish, insititious.

Basidiospores 8.0–10.0 × 4.5–5.0 µm [χ_m= 9.3± 0.7 × 4.8± 0.3 µm; Q_m= 1.9± 0.2; n=14], lacrimoid to ellipsoid, thin-walled, hyaline, inamyloid. *Basidia* clavate, 4-sterigmate, thin-

walled, inamyloid. *Basidioles* clavate and fusoid, thin-walled, inamyloid. *Cheilocystidia Siccus*-type, similar to pileocystidia. *Pleurocystidia* absent. *Pileipellis* iregular to hymeniform. *Pileocystidia* short, main body 10 × 5 µm, *Siccus*-type. *Pileus trama* and *Lamellar trama* interwoven, inamyloid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, clustery, leaf petiole on the leaf litter at terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK498 (INPA 259472).

34- *Marasmius* "red3"

Figs. 35A and B

Pileus 1–2 mm diam, convex aplanate, umbilicate papilate, opaque, dull, velutinous red (963B21); *context* pale, thin. *Lamellae* attached to collar, distant, *L*= 8, *l*= 0. *Stipe* 14–22 × 0.5 mm, central, cylindrical, wiry, smooth, polished black to dark-brown, rising from node of rhizomorph similar to stipe, erect, not racemose.

Basidiospores not observed. *Basidia* not observed. *Basidioles* clavate, some fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* modified *Siccus*-type broom cells, setulae irregular, finger-like, thick-walled. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. *Pileocystidia* 15 × 6 µm main body, modified *Siccus*-type browm cells, most thick-walled, finger-like setulae, irregular, branched. *Pileus trama* and *Lamellar trama* parallel to irregular, inamyloid. *Clamp connections* not observed.

Habit, habitat and known distribution: marasmioid, clustery, leaf petiole on the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 22 May 2012, D.L.Komura & F.C.Batista, DLK627 (INPA 259401).

35- *Marasmius* cf. *rotalis* Berk. & Broome, Botanical Journal of the Linnean Society 14: 40 (1875).

[MB#156152]

Holotype: ?. **Type locality:** Sri Lanka (?).

Fig. 36

Pileus 5–10 mm diam, broadly convex aplanate, sulcate, umbilicate with brown papila, opaque, dull, velutinous cream-pale (FAF3E3), depressed area white; *context* pale,thin. *Lamellae* attached to collar, subdistant, *L*= 17, *l*= 0, cream. *Stipe* 30 × 1 mm, central, cylindrical, wiry, smooth, polished, black to dark-brown, insititious. *Basidiospores* not

observed. *Basidia* not observed. *Basidioles* fusoid, thin-walled, hyaline. *Cheilocystidia* few number, 20 × 15 µm main body *Rotalis*-type, similar to pileocystidia, but thin-walled. *Pleurocystidia* absent. *Pileipellis* hymeniform. *Pileocystidia* 20 × 15 µm main body, *Rotalis*-type, thick-walled. *Pileus trama* and *Lamellar trama* interwove, inamyloid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, "Envira Amarela" leaf on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Jun 2012, D.L.Komura & F.R. Araújo, DLK811 (INPA 259744).

Comments: This is species is similar to decription in Singer (1976). ITS analysis showed that is close related with *M. castellanoi*.

36- *Marasmius castellanoi* Singer, Flora Neotropica 17: 99 (1976)

[MB#317278]

Holotype: BAFC. **Type locality:** Brazil, Pará, Estância Pirelli.

Fig. 37

Pileus 4–10 mm diam, broadly convex aplanate, sulcate, umbilicate with orange papila, brown in age, opaque, dull, cream-pale (FAF3E3); *context* pale, thin. *Lamellae* attached to collar, subdistant, *L*= 15, *l*= 0, cream. *Stipe* 10–15 × 1mm, central, cylindrical, wiry, smooth, polished, base dark-brown, cream into apex, insititious.

Basidiospores not observed. *Basidia* not observed. *Basidioles* fusoid, thin-walled, hyaline, inamyloid. *Cheilocystidia* not observed. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. *Pileocystidia Rotalis*-type, main body 20–30 × 10–15 µm, thin-walled, hyaline. *Pileus trama* and *Lamellar trama* interwoven, weakly dextrinoid. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf on the leaf litter.

Material examined: Brazil, Amazonas State, São Gabriel da Cachoeira, 01 Apr. 2013, D.L.Komura, C.E. Zartman, O.F. Menezes, C. Cardoso B. Agapito & A. Mirupu, DLK1114 (INPA271944).

Comments: This specimen is according to Singer description (1976). We just obtained one good sequence, other sequences from this species presented with many ambiguous sequences.

37- *Marasmius scleronematis* Singer, Fieldiana Botany 21: 54 (1989)

[MB#124936]

Holotype: INPA. **Type locality:** Brazil, Amazonas, 30 km N of Manaus.

Fig. 38

Pileus 3–5 mm diam, convex, sulcate, umbilicate with pale to dark-brown papila, opaque, dull, velutinous cream-pale (FAF3E3) to rust-brown (5C2F00,) with white to pale zone around the papilla; *context* pale, thin. *Lamellae* attached to collar, subdistant, *L*= 19–22, *l*= 0, cream to pale cream or brown as pileus. *Stipe* 12–20 × 1 mm, central, cylindrical, wiry, smooth, polished, dark-brown, cream to whitish, varing according to the age of the basidiomes, insititious.

Basidiospores 6.3–8.0 × 3.0–3.5 µm [χ_m= 6.9±0.5 × 3.2±0.2 µm; Q_m= 2.1±0.2; n=6], lacrimoid to ellipsoid, thin-walled, hyaline, inamyloid. *Basidia* not observed. Basidioles 20 × 6 µm, clavate, fusoid. *Cheilocystidia* composed by *Rotalis*-type broom cells. *Pleurocystidia* absent. *Pileipellis* irregular to hymeniform. *Pileocystidia Rotalis*-type broom cells. *Pileus trama* and *Lamellar trama* interwoven. *Clamp connections* present.

Habit, habitat and known distribution: marasmioid, close, leaf litter at terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 22 Aug. 2012, D.L.Komura, DLK881 (INPA 259626); 24 Aug. 2012, D.L.Komura, DLK891 (INPA 259627); 11 Sep. 2013, D.L.Komura & J.R. Maciel, DLK1756 (INPA271959).

Comments: This species was described from Manaus, Amazonas on leaves of *Scleronema micrantha* (Singer 1989). Due its great variation color, from cream to browm, it was very difficulty do indentify as the same species, just the ITS sequences showed that were the same species with high support the three sequences (100% BS).

Conclusions

The inclusion of ITS to morphological descriptions in *Marasmius* was usefull to species delimitation. However to better understand the evolutionary relationship among sections and subsection in *Marasmius* is necessary inclusion of others sequences and additional methods of analysis.

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Legends

Table S1. Strains and GenBank accessions of ITS sequences used in this study.

Fig. 1 *Marasmius* ssp. molecular phylogenetic analysis by Maximum Likelihood method based in ITS sequences. The bootstrap percentage > 70% is shown next to the branches. Branches in green represent one species.

Fig. 2A *Marasmius "*orange8". A, F- basidiomes; B- immature basidiome; C- pilear surface; D- detail the stipe with strigose base; E, G- lamellar view. Scale bar: 20 mm.

Fig. 2B Microscopic features of *Marasmius "*orange8". A- lamellar edge, showing cheilocystidia (top of the picture); B- interlamellar view, composed by pleurocystidia (left) and pilear surface with *Siccus*-type broom cells (right); C- lamellar and pilear trama in Melzer prep., showing strog dextrinoid reation (reddish brown color); D- Siccus-type broom cells on the pileus; E, G, F- Siccus-type broom cells, some thick-walled and setiform; G- Pleurocystidia fusoid to ventricose-rostrate and capitulate cells. Scale bars: A, B =50 µm; C= 60 µm; D, E, F, 20 µm; G, H= 10 µm.

Fig. 3A *Marasmius* "orange23". A- basidiomes; B- pileus surface; C- lamellae face. Scale bar: 10 mm.

Fig. 3B Microscopic features of *Marasmius* "orange23" in Melzer prep. A- basidiospores; B- pileocystidia, pilear trama and hymenium; C- lamellar trama; D, G- detail of the *Siccus*-type pileocystidia; E, F- *Siccus*-type cheilocystidia. Scale bar: 10 µm.

Fig. 4A *Marasmius suthepensis.* A- basidiomes; B- lamellae; C- pileus surface. Scale bar: 15 mm.

Fig. 4B Microscopic features of *Marasmius suthepensis.* A- basidiospores; B- capitulate pleurocystidia; C- lamellar edge, showing *Siccus-type* cheilocystidia (top); D- detail of the *Siccus*-type broom cells. Scale bars: A, D= 10 µm; C, B= 10 µm.

Fig. 5 Macroscopic and microscopic features of *Marasmius* "orange24". A- basidiome; B- pilear surface; C- lamellar face; D- pilear surface showing *Siccus*-type pileocsystidia; E- fusoid basidioles. Scale bars: A=8 mm; D, E= 20 µm.

Fig. 6A *Marasmius* "orange1". A- basidiomes; B- detail of the stipe with strigose base and immature basidiomes; C- pilear surface; D- lamellae view. Scale bar: 10 mm.

Fig. 6B Microscopic features of *Marasmius* "orange1". A- basidiospores; B- lamellar edge, showing *Siccus*-type cheilocystidia; C- *Siccus*-type pileocystidia. Scale bar: 10 µm.

Fig. 7 *Marasmius* "brown2". A- basidiomes; B-lamellae view. Scale bar: 5mm.

Fig. 8A *Marasmius* "orange9". A- basidiomes *in situ*; B, C- basidiomes; D, E, F- lamellar surface in different development stages; H,I= strigose mycelial base at the stipe. Scale bar: 10 mm.

Fig. 8B Microscopic features *Marasmius* "orange9". A, B- pileocystidia and pilear trama; C, G- *Siccus*-type pileocystidia; E- pilear surface view at top, showing *Siccus*-type pileocystidia; D- lamellar edge, showing *Siccus*-type cheilocystidia (bellow); F- *Siccus*-type pileocystidia. Scale bars: A, E, F= 30 µm; D, B= 60 µm; C, G= 20 µm.

Fig. 9A *Marasmius* cf. *griseoradiatus.* A, D- basidiomes; B- lamellar view; C- detail of the stipe with strigose basal mycelia. Scale bar: 10 mm.

Fig. 9B Microscopic features of *Marasmius* cf. *griseoradiatus.* A- basidiospores; B- lamellar edge, showing *Siccus*-type cheilocystidia; C- hymenial face (on the left) and pileae surface (on the right); D- lamellar view showing pleurocystidia in Melzer prep.; F- lamellar view showing pleurocystidia; G- *Siccus* type pleurocystidia; H- capitulate pleurocystidia. Scale bars: A=10 μm; B, C, D, F= 50 μm; E, H= 15 μm.

Fig. 10A *Marasmius* aff. *phaeus.* A- basiomes *in situ*; B- detail of sulcate pileus; C- lamellae view; D- detail from stipe with strigose basal mycelia. Scale bar 20 mm.

Fig. 10B Microscopic features of *Marasmius* aff. *phaeus.* A- lamellar edge, showing *Siccus*-type cheilocystidia; B, C, D- pilear layer, pilear trama and hymenium, showing Siccus-type pileocystidia. Scale bars: A, B, D= 20 μm; C= 50 μm.

Fig. 11A *Marasmius* "orange5". A- immature basidiome; B- lamellae; C, D- pilear surface, D- stipe base of immature and mature basidiome (on the right); E- mature basidiomes, F, G- lamellae view; H- strigose base of the stipe. Scale bar: 20 mm.

Fig. 11B Microscopic features of *Marasmius* "orange5". A- lamellar edge showing *Siccus*-type cheilocystidia; B- *Siccus*-type cystidia; C- pleurocystidia (on the right). Scale bar: 10 μm.

Fig. 12A *Marasmius haediniformis*. A- basidiomes *in situ*; B- stipe base; C, F- pileus shape; D- lamellar view; E- pileus surface. Scale bar: 10 mm.

Fig. 12B Microscopic features of *Marasmius haediniformis*. A- lamellar edge showing Siccus-type cheilocystidia; B- pleurocystidia as cystidiole, clavate and inconspicuous; C- Siccus-type cystidia; D- pilear view showing *Siccus*-type cheilocystidia; E- pleurocystidia as cystidiole, clavate and inconspicuous in Melzer prep. Scale bars: A, B, C= 10 μm.

Fig. 13A *Marasmius congregatus.* A- basidiomes *in situ*, B- pileus surface, C- lamellae face. Scale bar: 20 mm**.**

Fig. 13B Microscopic features of *Marasmius congregatus.* A, B- pilear view showing both *Globulares* and *Siccus*-type pileocystidia; C- stipetipellis showing caulocystidia; D- *Amyloflagelulla*-type caulocystidia; E- versiform caulocystidia. Scale bars: A, B, D, E= 20 μm**;** C= 70 μm.

Fig. 14 Macroscopic and microscopic features of *Marasmius* "orange10". A- basiomes, B- pileus; C- lamellae view; D- pilear surface showing *Siccus*-type pileocystidia; E- basidiospores; F- *Siccus*-type cystidia; Lamellar trama, dextrinoid reaction in Melzer prep. Scale bars: A= 10 mm; E, F= 10 μm.

Fig. 15A *Marasmius bellus.* A- basidiome; B- pileus surface; C- lamellar view. Scale bar: 15 mm.

Fig. 15B Microscopic features of *Marasmius bellus.* A- *Siccus*-type cheilocystidia; B- *Siccus*-type pileocystidia; C- pileocystidia. D- cheilocystidia; E- lamellar edge. Scale bars: C, D= 10 μm.

Fig. 16A *Marasmius* "orange2". A, B, C- different shapes of basidiomes; D, E, F- lamellar view; G- strigose basal mycelia from stipe, H, I- pilear surface. Scale bars: A, B, C, = 15 mm.

Fig. 16B Microscopic features of *Marasmius* "orange2". A- basidiospore; B- modified *Siccus*-type pileocystidia; C, E- caulocystidia; D- stipitipellis with caulocystidia. Scale bars: A, B, C, E= 10 μm; D= 50 μm.

Fig. 17A *Marasmius ruber.* A, F- basidiomes; B, E, H- lamellar suface; C, D- young basiomes, detail of the strigose base from stipe; G- pilear surface, old basidiome. Scale bar: 20 mm.

Fig. 17B Microscopic features of *Marasmius ruber.* A- hymenial view showing basidioles and basidiospores; B- lamellar edge showing *Siccus*-type. Scale bar A= 25 μm; B= 50 μm.

Fig. 18A *Marasmius* "orange4". A, D, E- basidiomes; B- lamellar face immature basidiome; C- strigose basal mycelia from stipe; F- lamellar face from old basidiome. Scale bar 20 mm.

Fig. 18B Microscopic features of *Marasmius* "orange4". A- basidiospores; B- lamellar trama and cheilocystidia in Melzer prep.; C- transversal cutting from lamellae and pileipelis in Melzer prep.; D- hymenial layer with basidiospores attached to basidium, E, F- pileocystidia. Scale bars: A= 10 μm; C= 100 μm.

Fig. 19A *Marasmius cladophyllus*. A, C- basidiomes; B, D- lamellar view; E- stipe base. Scale bar: 20 mm.

Fig. 19B Microscopic features of *Marasmius cladophyllus*. A- section of the lamella in Melzer prep.; B- lamellar edge showing *Siccus*-type cheilocystidia; C- basidiospore; D- *Siccus*-type pileocystidia; E- pileipelis and pilear trama in Melzer prep. showing dextrinoid reaction in Melzer prep. (reddish brown color). Scale bars: A= 70 μm; C= 10 μm; D= 15 μm.

Fig. 20A *Marasmius* cf. *digilioi.* A- basidiome; B- lamellar view; C- strigose base from stipe; D- pileus surface. Scale bar: 10 mm.

Fig. 20B Microscopic features of *Marasmius* cf. *digilioi.* A- pileipelis with *Siccus*-type cystidia; B- lamellar edge with *Siccus*-type cheilocystidia; C- stipitipellis with caulocystidia; D- caulocystidia. Scale bar: A, D= 10 μm.

Fig. 21A *Marasmius* cf. *trinitatis.* A- basidiome; B- pileus surface; C- strigose base from stipe; D- lamellae view. Scale bar 10 mm.

Fig. 21B Microscopic features of *Marasmius* cf. *trinitatis*. A- section showing pileipelis, pileus trama; B- lamellar edge showing *Siccus*-type cheilocystidia; C, D- stipitipellis with caulocystidia; E- stipitipellis showing detail of the clamp connections. Scale bar: A= 10 μm.

Fig. 22A *Marasmius phaeus*. A, C- basidiomes; B- pileus; D- lamellae view, E- strigose stipe base. Scale bar 10 mm.

Fig. 22B *Marasmius phaeus*. A- basidiospores; B- basidiopores attached to basidium; C, D- cystidia. Scale bars: A, B, C, D= 10 μm.

Fig. 23A *Marasmius hypophaeus*. A- basidiomes; B- lamellae view. Scale bar 20 mm.

Fig. 23B Microscopic features of *Marasmius hypophaeus*. A- section showing pileipelis and lamella; B- lamelar edge showing *Siccus*-type cheilocystidia; C- basidiospores; D- hymenial layer showing basidia and incosnpicuous pleurocystidia; E, F- basidia; G- *Siccus*-type cystidia. Scale bar: C, D, G= 10 μm.

Fig. 24A *Marasmius haematocephalus*. A, D- basidiomes; B, E- pileus; E, F- lamellae. Scale bar: 10 mm.

Fig. 24B Microscopic features of *Marasmius haematocephalus*. A- lamellar section showing pleirocystidia; B- lamellar section showing pleirocystidia in Melzer prep. (reddish brown color showing strong dextrinoid reaction); C- *Siccus*-type pileocystidia in Melzer prep.; D- fusoid pleurocystidia. Scale bar: A, D= 20 μm.

Fig. 25A *Marasmius berteroi*. A- basidiome; B- lamellae view; C- pilear surface. Scale bar 10 mm.

Fig. 25B Microscopic features of *Marasmius berteroi*. A- basidiospores; B- *Siccus*-type pleurocystidia; C- *Siccus*-type pleurocystidia and 4-sterigmate basidium (on the right); D- immature basidiospores attached to basidium; E, F- *Siccus*-type pileocystidia, some thick-walled. Scale bar: A= 20 μm.

Fig. 26 Macroscopic and microscopic features of *Marasmius* "orange7". A- basidiomes; B- detail umbilicate pileus; C- lamellae view; D- basidisopore; E- *Siccus*-type pileocystidia. some modified and thick-walled. Scale bars: A= 5 mm; E= 10 μm.

Fig. 27A *Marasmius guyanensis*. A, B- basidiomes; C- pilear surface; D- lamellae. Scale bar: 20 mm.

Fig. 27B Microscopic features of *Marasmius guyanensis*. A- lamellar section showing *Siccus*-type cheilocystidia in Melzer prep.; B- pilear section showing *Siccus*-type pileocystidia in Melzer prep.; C- *Siccus*-type pileocystidia. Scale bar: C= 10 μm.

Fig. 28A *Marasmius tageticolor*. A- immature basidiomes; B, C- basiomes; D- pileus suface from young basidiome and F- from old; G- lamellar face from young basidiome and E, H- mature basidiomes, I- stipe base at substrate. Scale bars: A, C=10 mm.

Fig. 28B Microscopic features of *Marasmius tageticolor.* A- basidiopores; B- hymenium and a basidia; C, D- *Siccus*-type pileocystidia; E- *Siccus*-type cheilocystidia. Scale bars: 10 μm.

Fig. 29A *Marasmius lilacinoalbus*. A- pileus; B- lamellae view; C- basidiomes. Scale bar 20 mm.

Fig. 29B Microscopic features of *Marasmius lilacinoalbus*. A- basidiospores; B,C- basidia; D- pileipellis composed by *Siccus*-type pileocystidia; E- lamellar edge with Siccus-type cheilocystidia in Melzer prep. Scale bars: A, B, C= 10 μm.

Fig. 30A *Marasmius leoninus*. A- basidiomes *in situ*; B,D- lamellae view; C- detail of the mycelial pad. Scale bar: 20 mm.

Fig. 30B Microscopic features of *Marasmius leoninus*. A- section of the lamella end pileus; B,C- basidiospores; D- *Siccus*-type pileocystidia; E- hymenial section showing a basidium; F,G- Siccus-type cheilocystidia; H- lamellar edge showins cheilocystidia. All pictures showed are in Melzer prep. Scale bar: 30 10 μm.

Fig. 31A *Marasmius* "orange21". A, H- basidiomes; B, C- pilear surface; D, E, F- lamellar view; G- lamellar view from young basidiome, J, K- immature basidiomes, I- detail from stipe base. Scale bar 20 mm.

Fig. 31B Microscopic features of *Marasmius* "orange21". A- basidiospores; B, C, D- *Siccus*-type cystidia. Scale bar: 10 μm.

Fig. 32A *Marasmius jalapensis.* A- basidiome; B- pilear surface; C- pruinous stipe with strigose base, D- lamellae view. Scale 20 mm.

Fig. 32B Microscopic features of *Marasmius jalapensis.* A- basidiome; B- pilear surface; C- pruinous stipe with strigose base, D- lamellar view. Scale 50 μm.

Fig. 33 Macroscopic and microscopic features of *Marasmius* "orange3". A- basidiomes; B- lamellae attached to collar; C- *Siccus*-type cheilocystidia, some modified type. Scale bars: 1= 5 mm; C, D= 15 μm.

Fig. 34 Macroscopic and microscopic features of *Marasmius* "orange16". A- basidiomes; B- pileus; C- lamellae attached to collar; D- basidiospores; E- pileipellis; F- lamellar edge showing *Siccus*-type cheilocystidia. Scale bar: A= 10 mm; D= 10 μm.

Fig. 35A *Marasmius* "red3". A- basidiomes; B, C- basidiome attached to rhizomorph; D- lamellae attached to collar. Scale bar 5 mm.

Fig. 35B Microscopic features of *Marasmius* "red3". A- lamellar edge showing *Siccus*-type cheilocystidia; B- *Siccus*-type cystidia, some thick-walled; C- pileocystidia. Scale bar: 10 μm.

Fig. 36 Macroscopic and microscopic features of *Marasmius rotalis*. A, B- basidiomes; C- lamellae attached to collar; D- pileus umbilicate and papilate; E- pilear section showing *Rotalis*-type pileocystidia; F- *Rotalis*-type pileocystidia. Scale bar: A= 10 mm; F= 10 μm.

Fig. 37 Macroscopic and microscopic features of *Marasmius castellanoi.* A- basidiomes; B- lamellae attached to collar; C- pilear section showing *Rotalis*-type pileocystidia; D- *Rotalis*-type pileocystidia. Scale bar: A= 10 mm; D= 10 μm.

Fig. 38 *Marasmius scleronematis.* A, G- lamellae attached to collar, B, C, F- basidiomes, D, E- pileus umbilicate and papilate, H- sulcate pileus. Scale bar 5 mm.

Table S1. Strains and GenBank accessions of ITS sequences used in this study.

Species	**collection ID**	**Herbarium accession No.**	**Location**	**Genbank accession No.**	**References**
Marasmius "orange8"	DLK1226	INPA259479	AM, Brazil		this work
M. "orange8"	DLK616	INPA259450	AM, Brazil		this work
M. "orange8"	DLK1201	INPA259452	AM, Brazil		this work
M. "orange8"	DLK1456	INPA259454	AM, Brazil		this work
M. suthepensis	JO293		SP, Brazil		Oliveira 2014
M. "orange23"	DLK1239	INPA259488	AM, Brazil		this work
M. "orange23"	DLK634	INPA271938	AM, Brazil		this work
M. suthepensis	TYS280		Thailand	EU935520	Schoch et al. 2011
M. cf. *suthepensis*	DLK1844	INPA271966	AM, Brazil		this work
M. suthepensis	JO329		SP, Brazil		Oliveira 2014
M. suthepensis	JO469		SP, Brazil		Oliveira 2014
M. "orange24"	DLK1483	INPA259493	AM, Brazil		this work
M. "orange1"	DLK499	INPA259449	AM, Brazil		this work
M. "brown2"	DLK500	INPA259631	AM, Brazil		this work
*M. "*orange9	DLK1894	INPA259460	PA, Brazil		this work
M. "orange9"	DLK1942	INPA259461	PA, Brazil		this work
M. cf. *griseoradiatus*	DLK1939	INPA259737	PA, Brazil		this work
M. aff. *phaeus*	DLK1895	INPA259376	PA, Brazil		this work
M. "orange5"	DLK1359	INPA259492	PA, Brazil		this work
M. "orange5"	DLK1580	INPA271957	AM, Brazil		this work
M. haediniformis	DLK502	INPA271934	AM, Brazil		this work
M. congregatus	JO468		SP, Brazil		Oliveira 2014
M. congregatus	JO122		SP, Brazil		Oliveira 2014
M. congregatus	DLK1866	INPA271970	PA,Brazil		this work
*M. "*orange10"	DLK1299	INPA259464	AM, Brazil		this work
M. bellus	DLK1736	INPA259505	AM, Brazil		this work
M. bellus	JO299		SP, Brazil		Oliveira 2014
M. "orange2"	DLK676	INPA259435	AM, Brazil		this work
M. "orange2"	DLK1843	INPA271965	AM, Brazil		this work
M. "orange2"	DLK892	INPA271941	AM, Brazil		this work
M. ruber	DLK1966		PA, Brazil		this work
M. ruber	DED8669		SP, Brazil		Oliveira 2014
M. "orange4"	DLK1725	INPA259738	AM, Brazil		this work
M. "orange4"	DLK1356	INPA271949	AM, Brazil		this work
M. cladophyllus	DLK1192	INPA271945	AM, Brazil		this work
M. cladophyllus	DLK1256	INPA259739	AM, Brazil		this work
M. cladophyllus	DLK506	INPA271935	AM, Brazil		this work
M. cladophyllus	DLK1464	INPA259740	AM, Brazil		this work
M. cladophyllus	DLK298		AM, Brazil		this work

M. digilioi	DLK1826	INPA271961	AM, Brazil		this work
M. trinitatis	JO306		SP, Brazil		Oliveira 2014
M. trinitatis	DLK1896	INPA271971	PA, Brazil		this work
M. phaeus	DLK326	INPA271929	AM, Brazil		this work
M. phaeus	DLK1472	INPA271954	AM, Brazil		this work
M. phaeus	DLK412	INPA271931	AM, Brazil		this work
M. phaeus	DLK882	INPA259375	AM, Brazil		this work
M. phaeus	DLK485	INPA271933	AM, Brazil		this work
M. hypophaeus	JO454		SP, Brazil		Oliveira 2014
M. hypophaeus	JO387		SP, Brazil		Oliveira 2014
M. hypophaeus	JO438		SP, Brazil		Oliveira 2014
M. hypophaeus	DLK1833	INPA271963	AM, Brazil		this work
M. haematocephalus	NW2008b		Thailand	EU935534	Schoch et al. 2011
M. haematocephalus	NW2008c		Thailand	EU935536	Wannathes et al. 2009
M. haematocephalus	NW2008a		Thailand	EU935525	Wannathes et al. 2009
M. haematocephalus	DLK1579	INPA271956	AM, Brazil		this work
M. haematocephalus	DED8675		SP, Brazil		Oliveira 2014
M. haematocephalus	JO533		SP, Brazil		Oliveira 2014
M. haematocephalus	JO282		SP, Brazil		Oliveira 2014
M. rhabarinus	JO474		SP, Brazil		Oliveira 2014
M. rhabarinus	JO533		SP, Brazil		Oliveira 2014
M. rhabarinus	JO282		SP, Brazil		Oliveira 2014
M. berteroi	DLK1849	INPA271967	AM, Brazil		this work
M. rhabarinoides	JO066		SP, Brazil		Oliveira 2014
M. orange7	DLK1276	INPA259474	AM, Brazil		this work
M. cf. *guyanensis*	DLK1832	INPA271962	AM, Brazil		this work
M. cf. *guyanensis*	DLK1837	INPA271964	AM, Brazil		this work
M. tageticolor	DLK508	INPA271936	AM, Brazil		this work
M. tageticolor	DLK1746	INPA271958	AM, Brazil		this work
M. tageticolor	DLK1369	INPA271950	AM, Brazil		this work
M. tageticolor	DLK1399	INPA271951	AM, Brazil		this work
M. lilacinoalbus	DLK1932	INPA259383	PA,Brazil		this work
M. leoninus	DLK1215	INPA259478	AM, Brazil		this work
M. leoninus	DLK591	INPA271937	AM, Brazil		this work
*M. "*orange21"	DLK1249	INPA271947	AM, Brazil		this work
*M. "*orange21"	DLK1241	INPA259489	AM, Brazil		this work
*M. "*orange21"	DLK690	INPA271939	AM, Brazil		this work
*M. "*orange21"	DLK1217	INPA271946	AM, Brazil		this work
*M. "*orange21"	DLK1413	INPA271953	AM, Brazil		this work
M. atrorubens	JO489		SP, Brazil		Oliveira 2014
M. atrorubens	JO528		SP, Brazil		Oliveira 2014
M. cf. *jalapensis*	DLK1944	INPA271973	PA,Brazil		this work
M. anomalus	JO346		SP, Brazil		Oliveira 2014
*M. "*orange3"	DLK1338	INPA259438	AM, Brazil		this work
M. guyanensis	JO345		SP, Brazil		Oliveira 2014
M. guyanensis	JO344		SP, Brazil		Oliveira 2014

*M. "*orange16"	DLK498	INPA259472	AM, Brazil		this work
*M. "*red3"	DLK627	INPA259401	AM, Brazil		this work
M. rotula	AW274	PBM 2563	?	DQ182506	Matheny;Hibbett 2005
M. rotula	S31		Germany	JN714927	Schoch et al. 2011
M. rotula	NN005958		Denmark	JN943598	Schoch et al. 2011
M. rotalis	DLK811	INPA259744	AM, Brazil		this work
M. castellanoi	DLK1114	INPA271944	AM, Brazil		this work
M. scleronematis	DLK881	INPA259626	AM, Brazil		this work
M. scleronematis	DLK891	INPA259627	AM, Brazil		this work
M. scleronematis	DLK1756	INPA271959	AM, Brazil		this work

Fig. 1 (first parte)

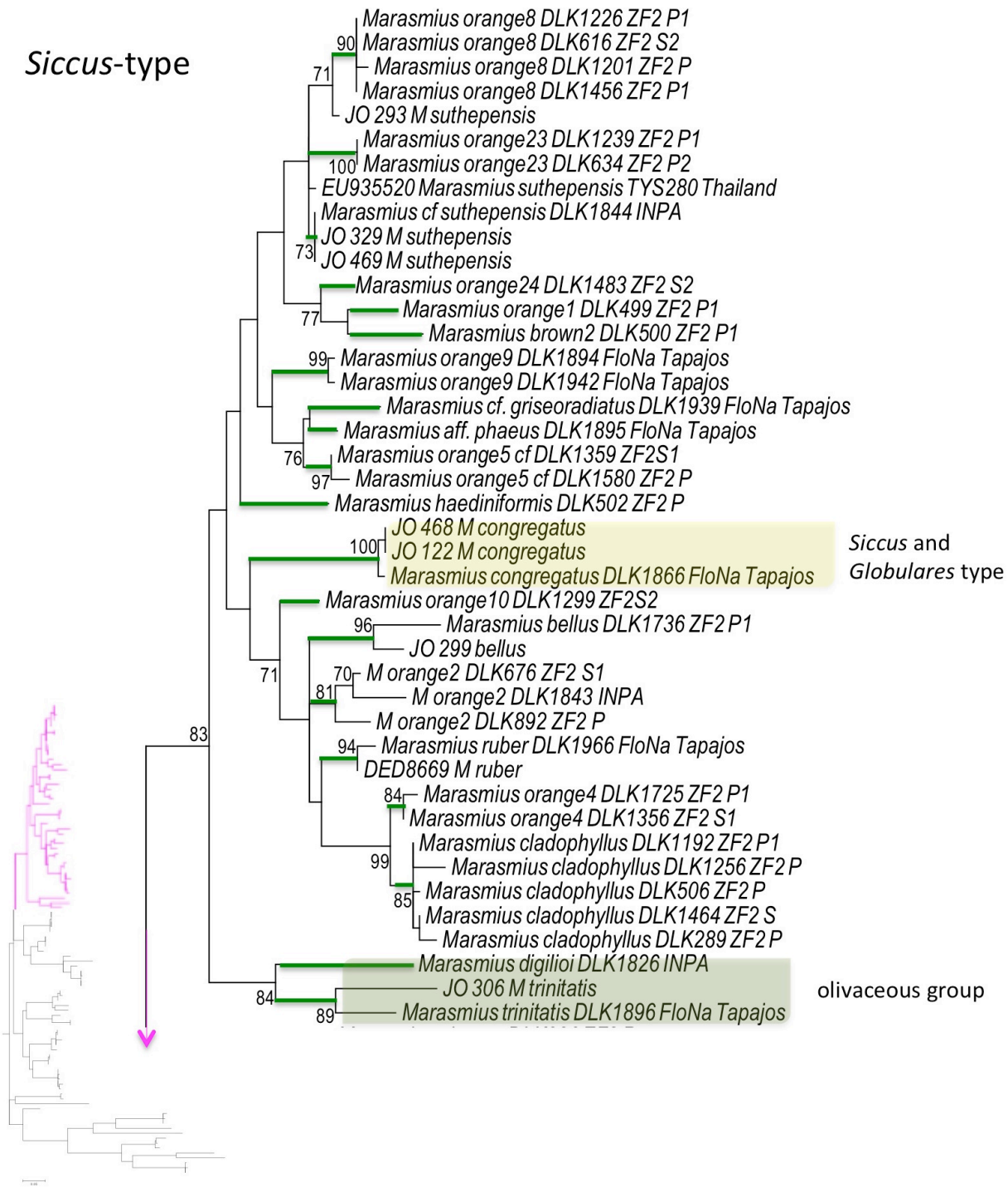


Fig. 1 (second part)

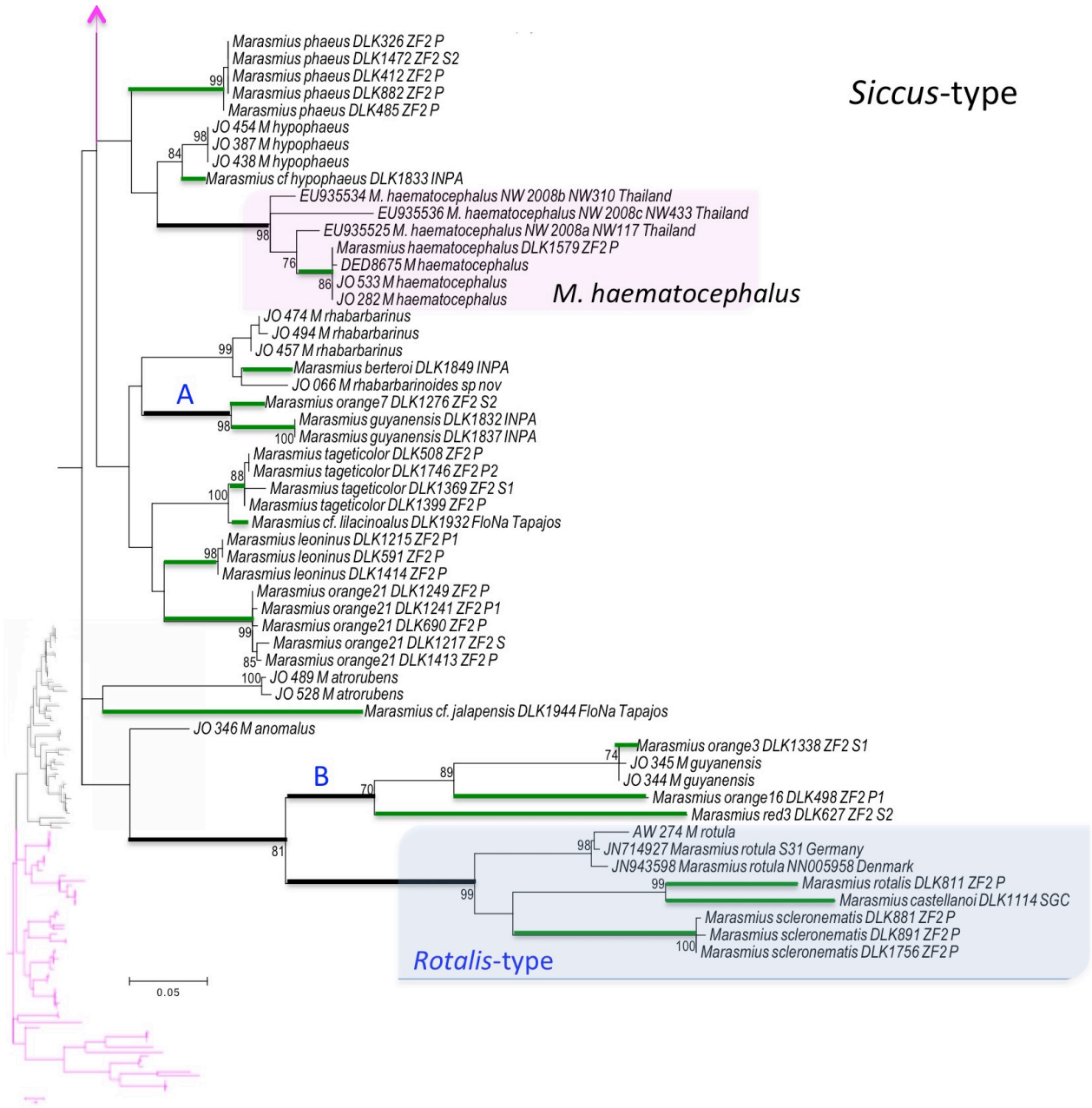


Fig. 2A ***Marasmius*** **"orange8"**

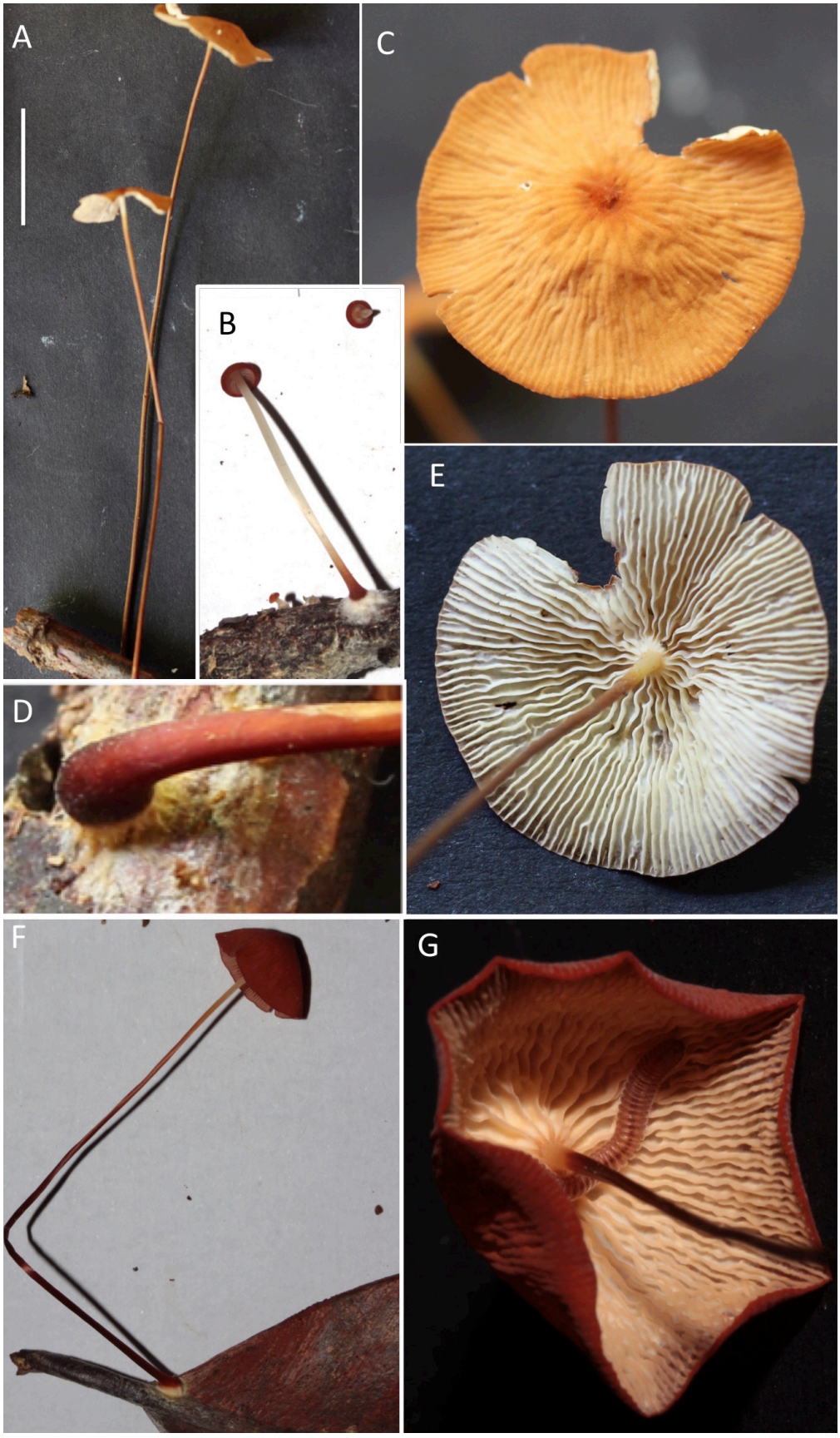


Fig. 2B ***Marasmius*** **"orange8"**

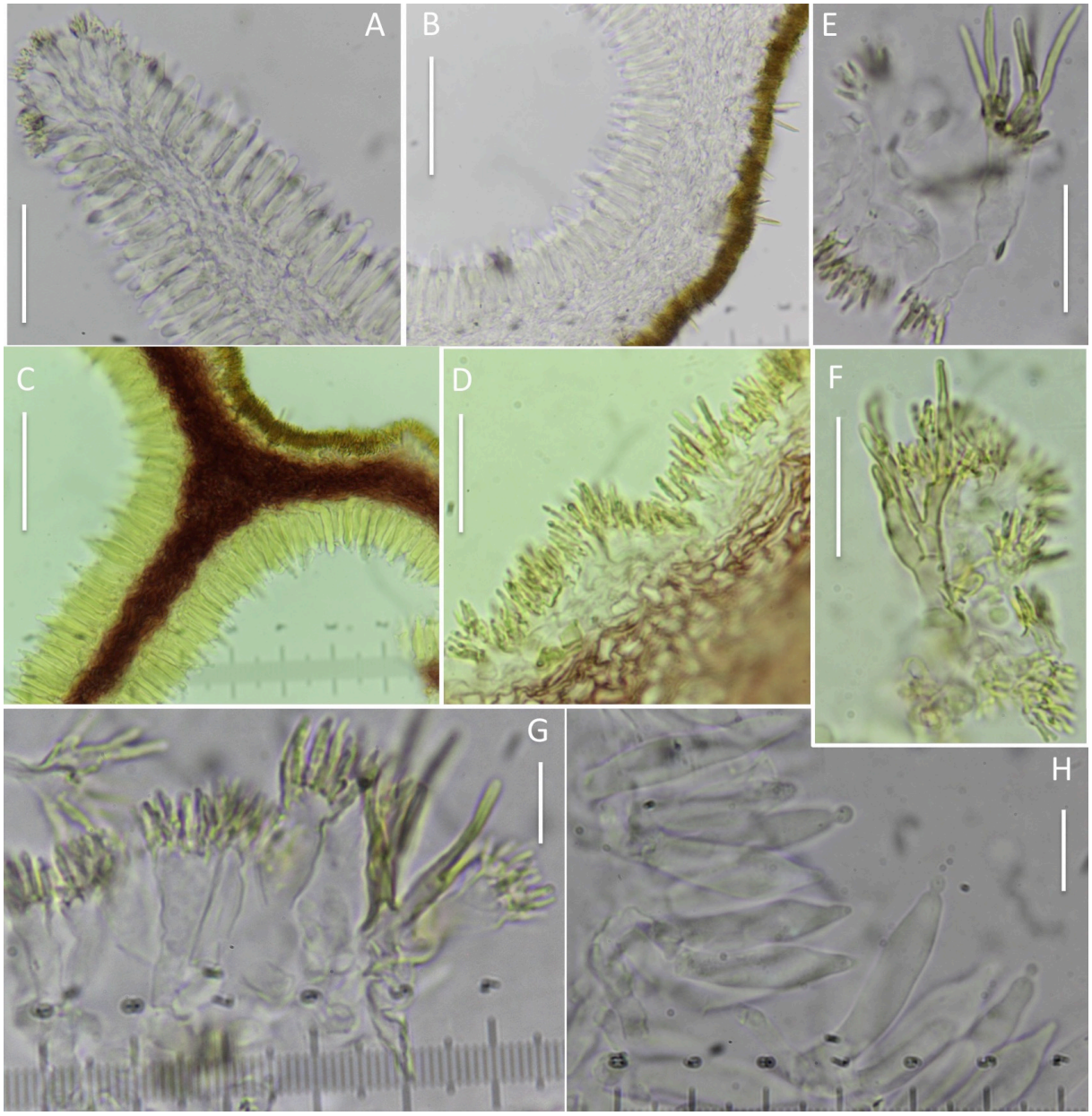


Fig. 3A ***Maramius*** **"orange23"**

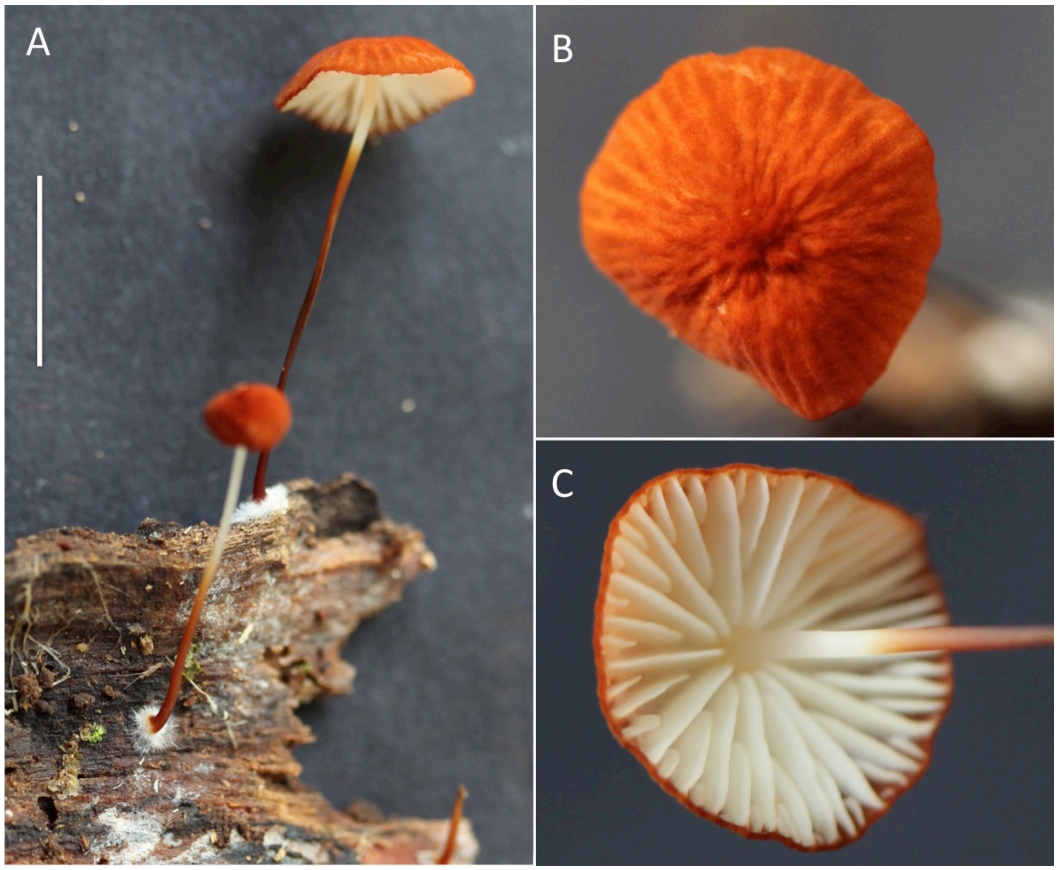


Fig. 3B ***Maramius*** **"orange23"**

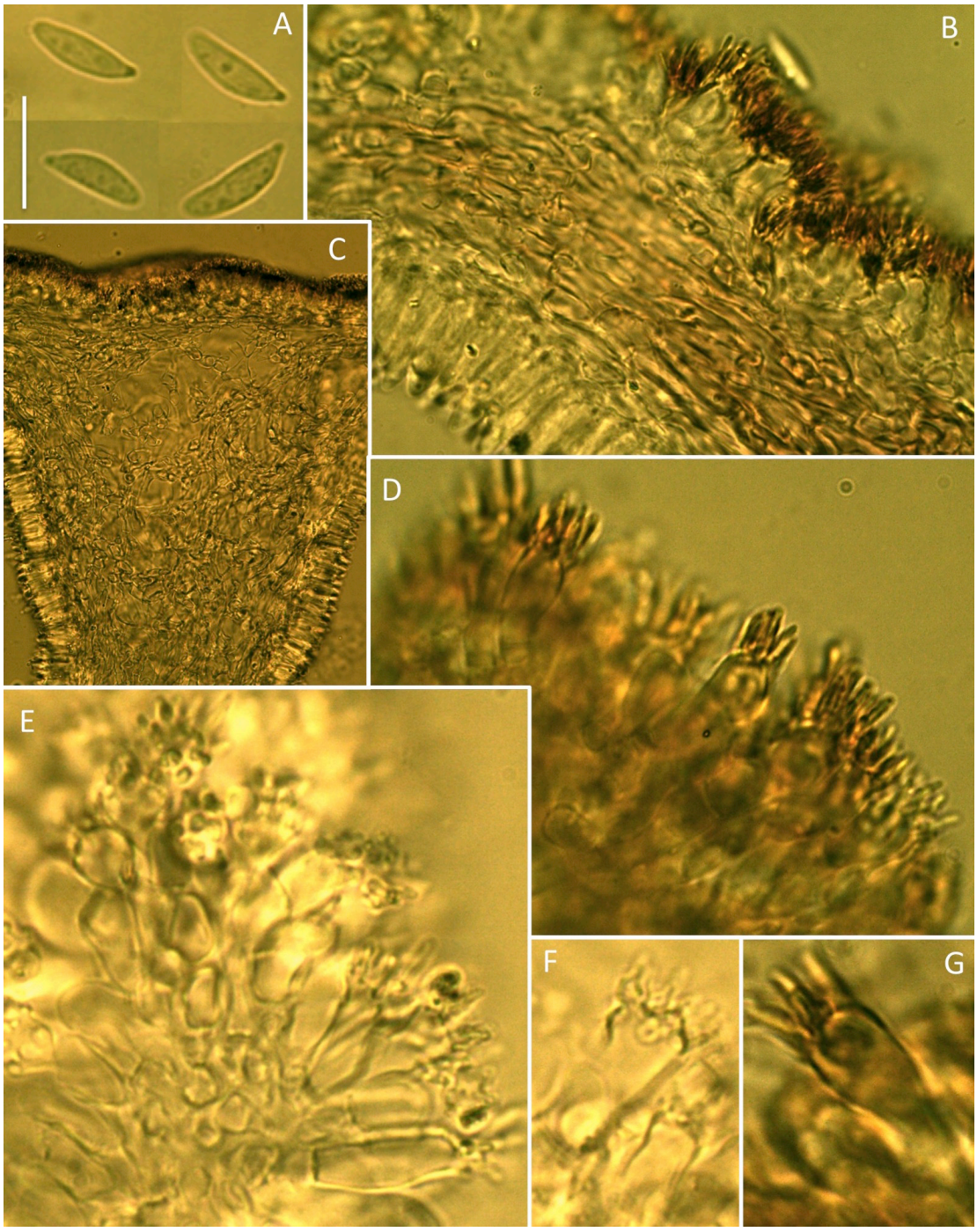


Fig. 4A ***Marasmius suthepensis***

Fig. 4B ***Marasmius suthepensis***

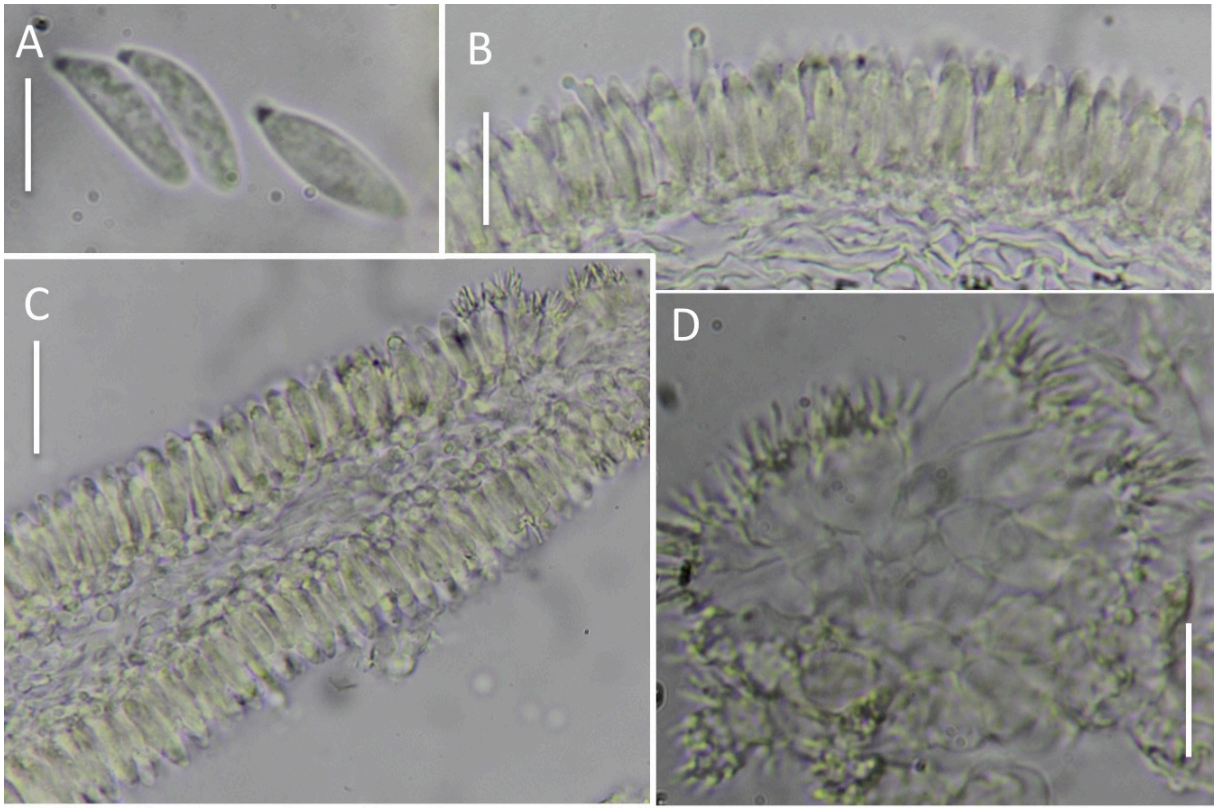


Fig. 5 ***Marasmius*** **"orange24"**

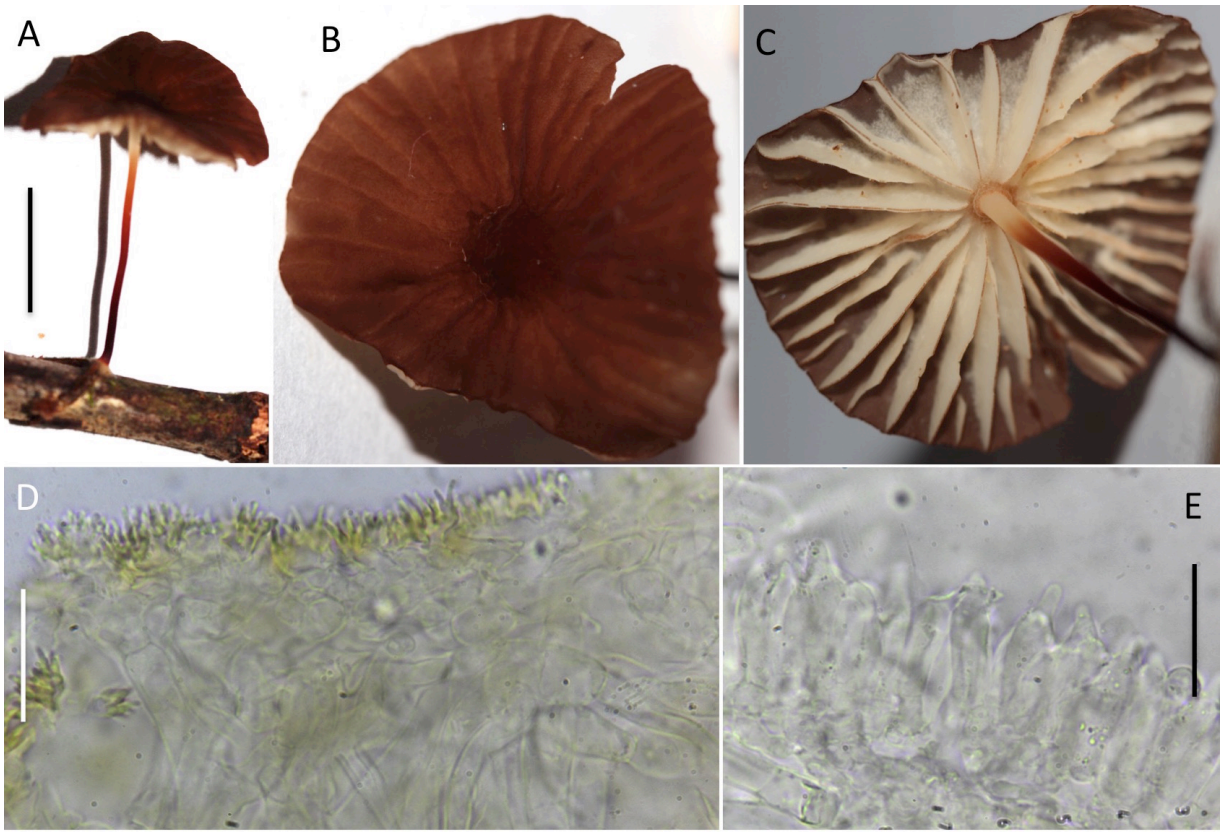


Fig. 6A ***Marasmius*** **"orange1"**

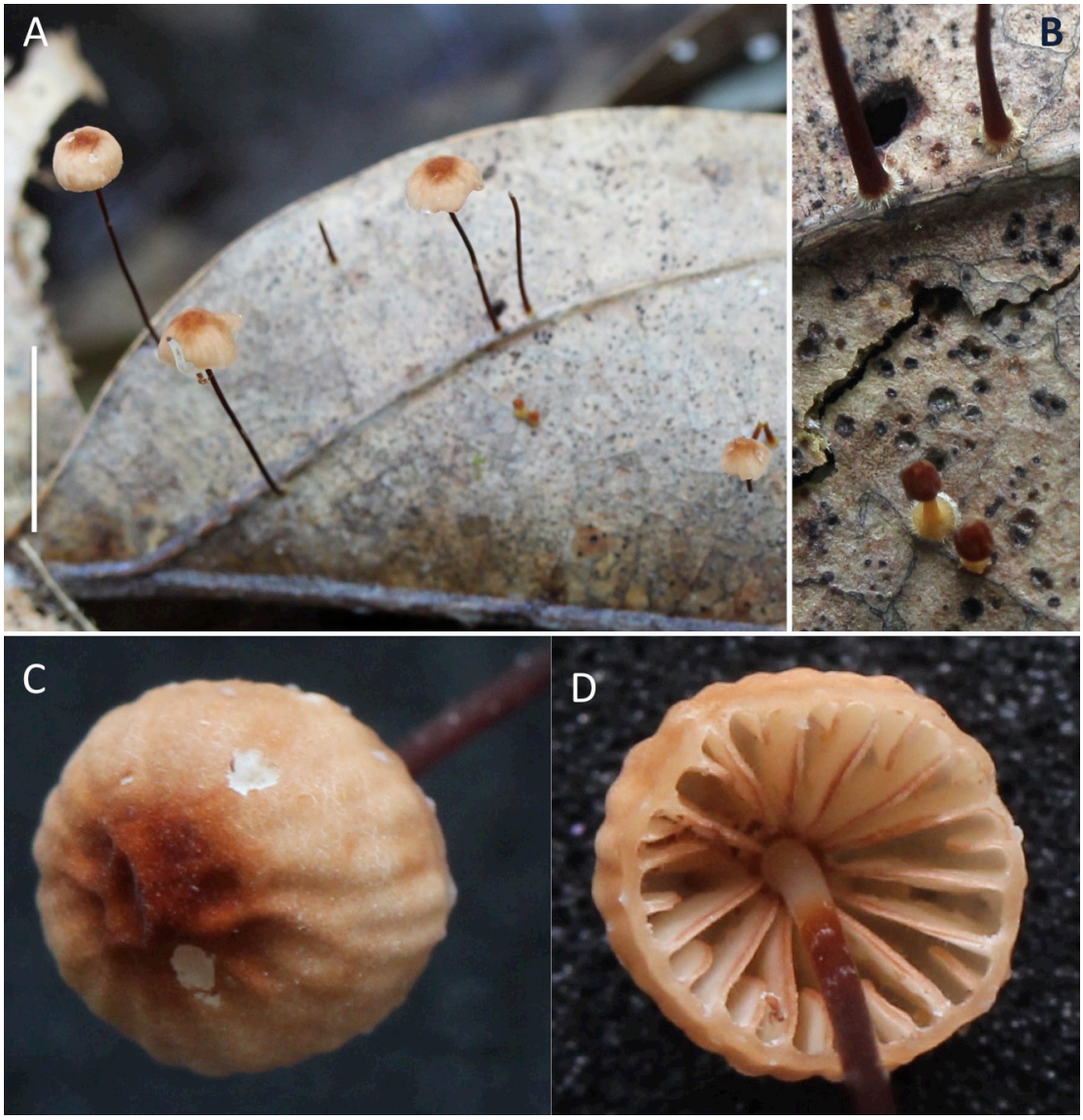


Fig. 6B *Marasmius* "orange1"

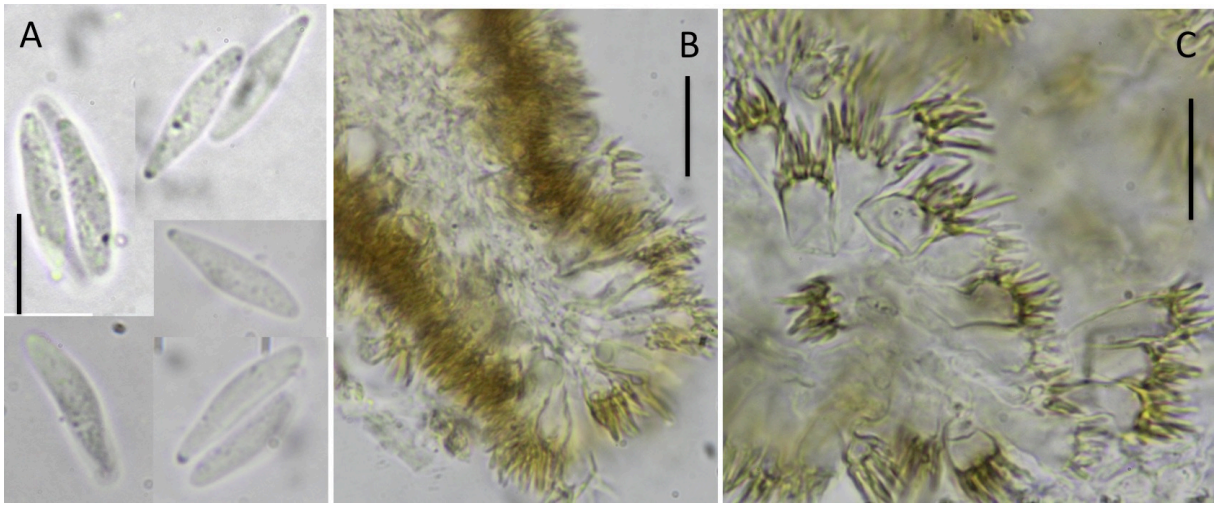


Fig. 7 ***Marasmius*** **"brown2"**

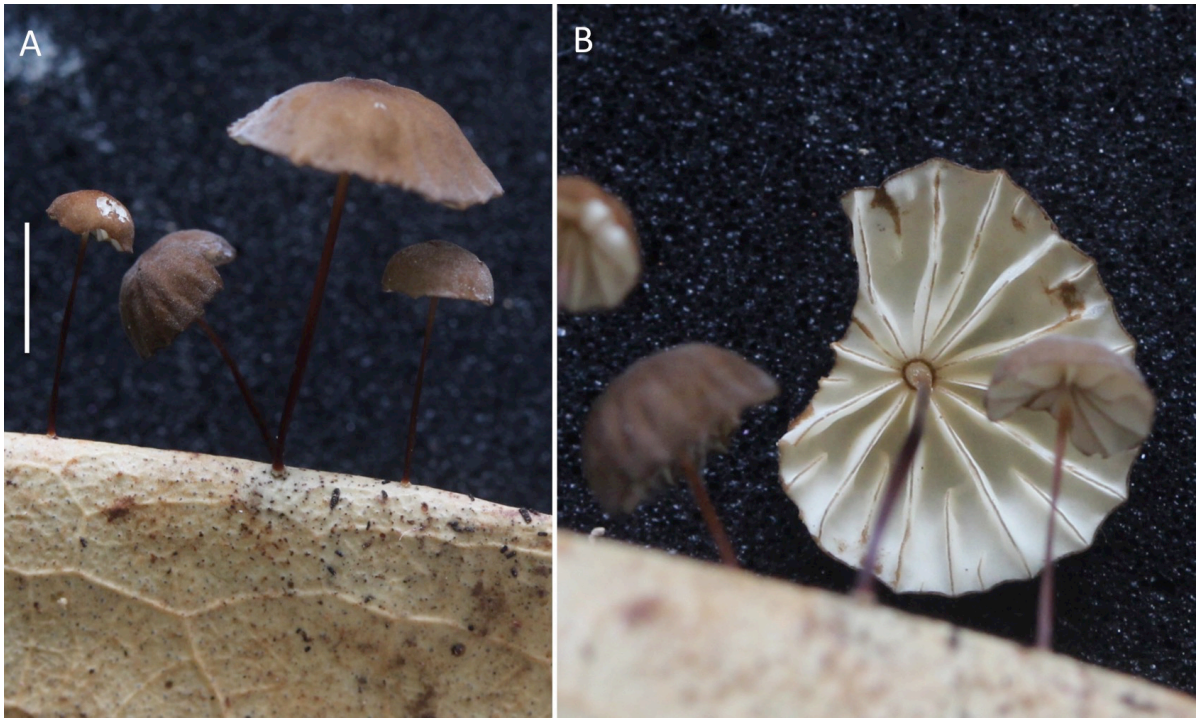


Fig. 8A ***Marasmius*** **"orange9"**

Fig. 8B ***Marasmius*** **"orange9"**

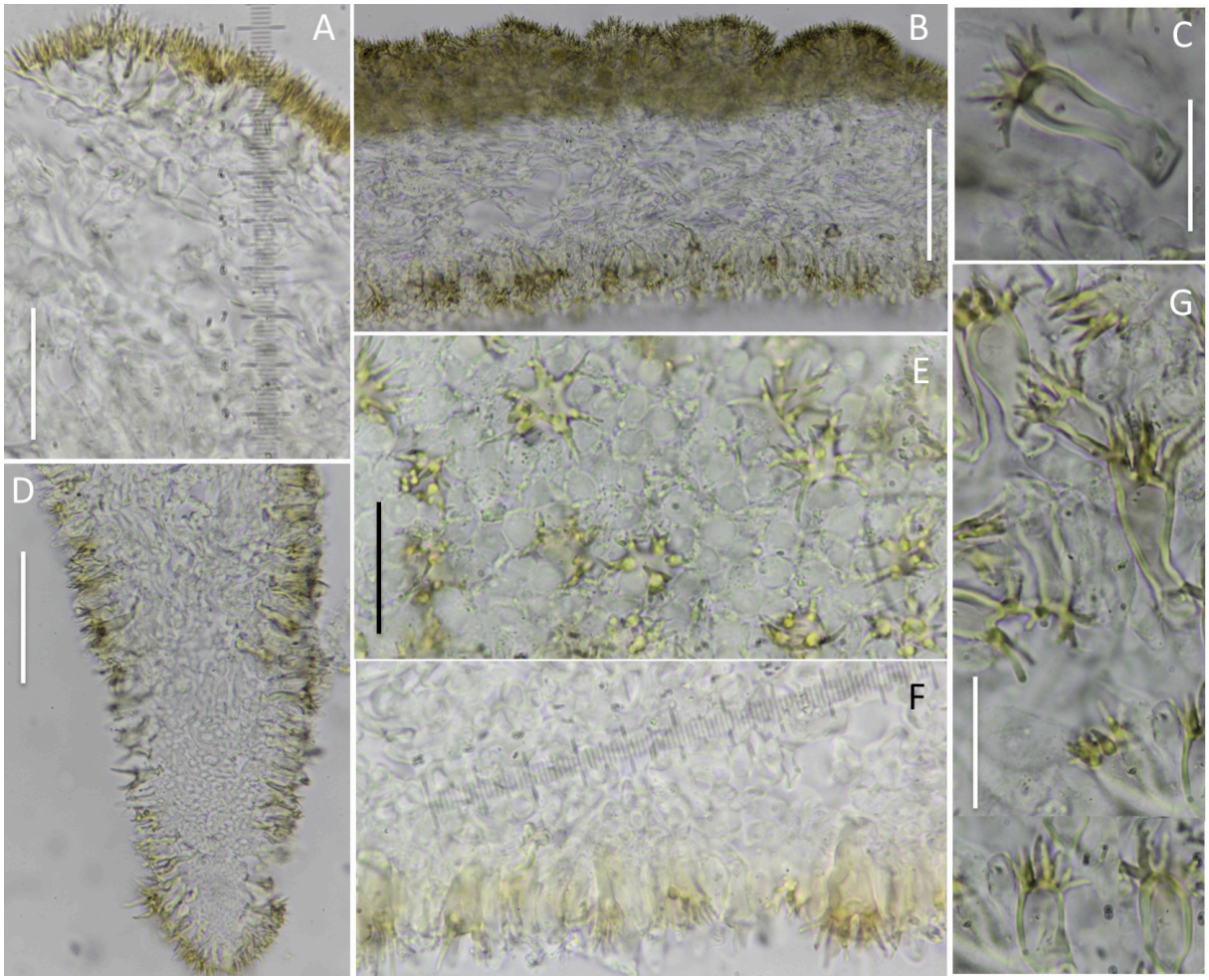


Fig. 9A ***Marasmius*** **cf.** ***griseoradiatus***

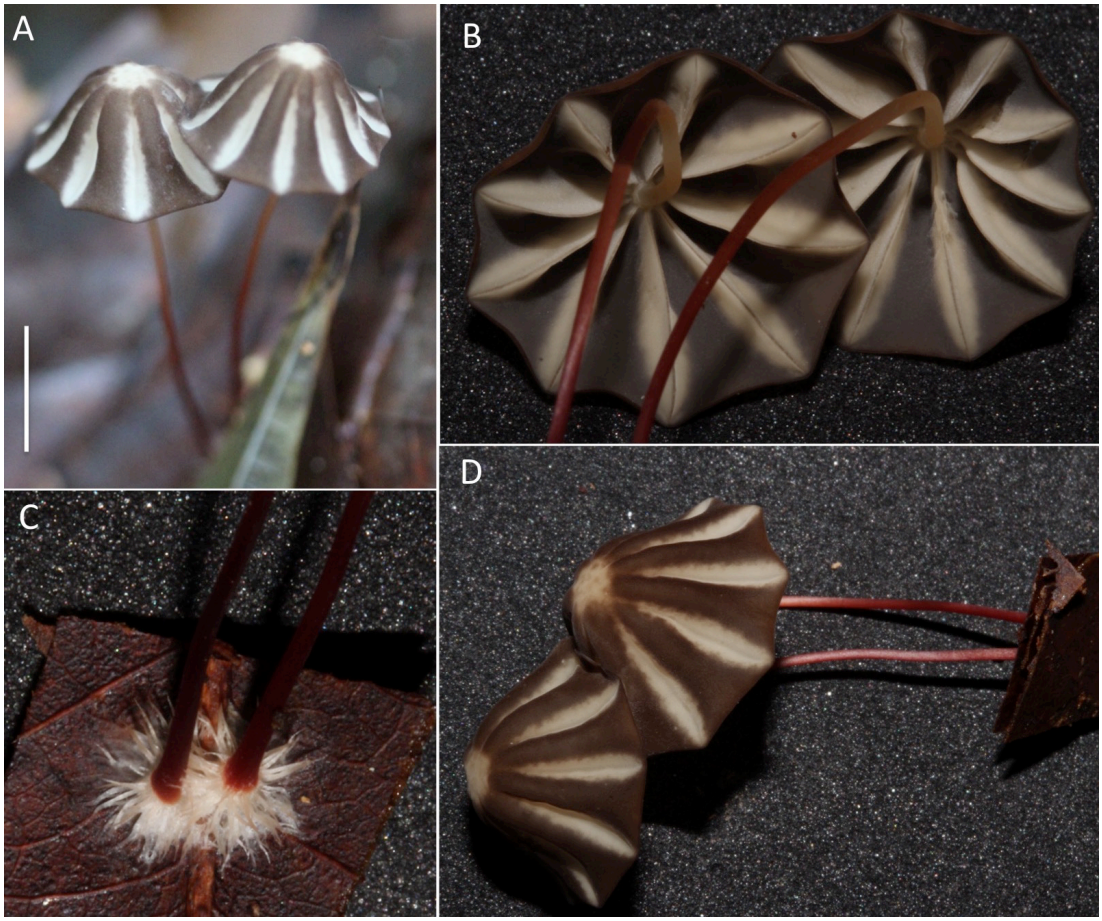


Fig. 9B ***Marasmius*** **cf.** ***griseoradiatus***

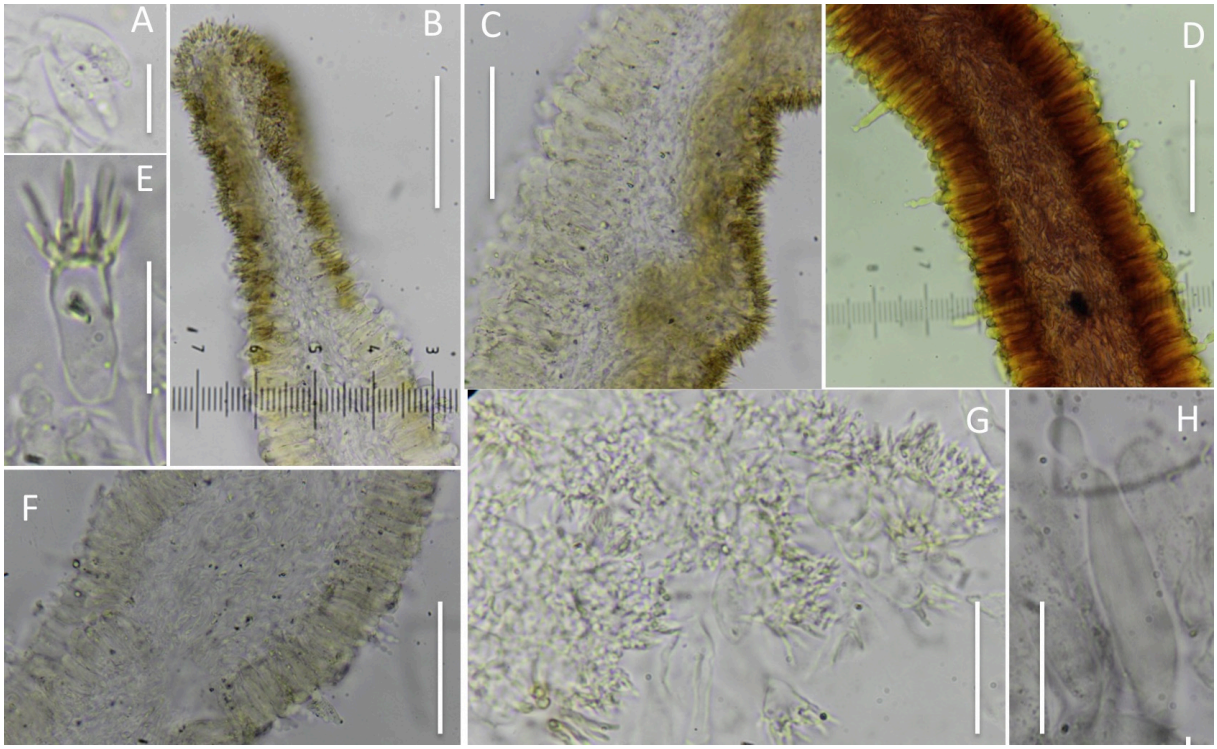


Fig. 10A ***Marasmius*** **aff.** ***phaeus***

Fig. 10B ***Marasmius*** **aff.** ***phaeus***

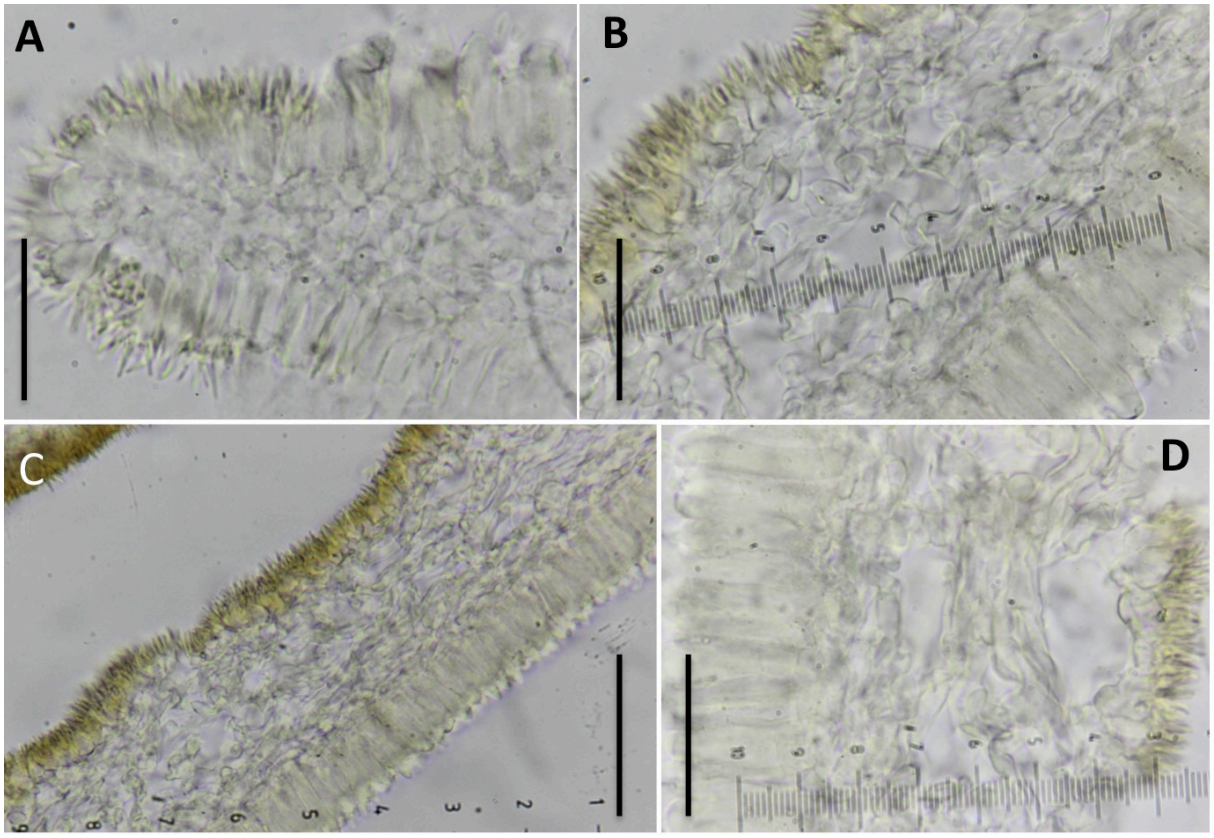


Fig. 11A ***Marasmius*** **"orange5"**

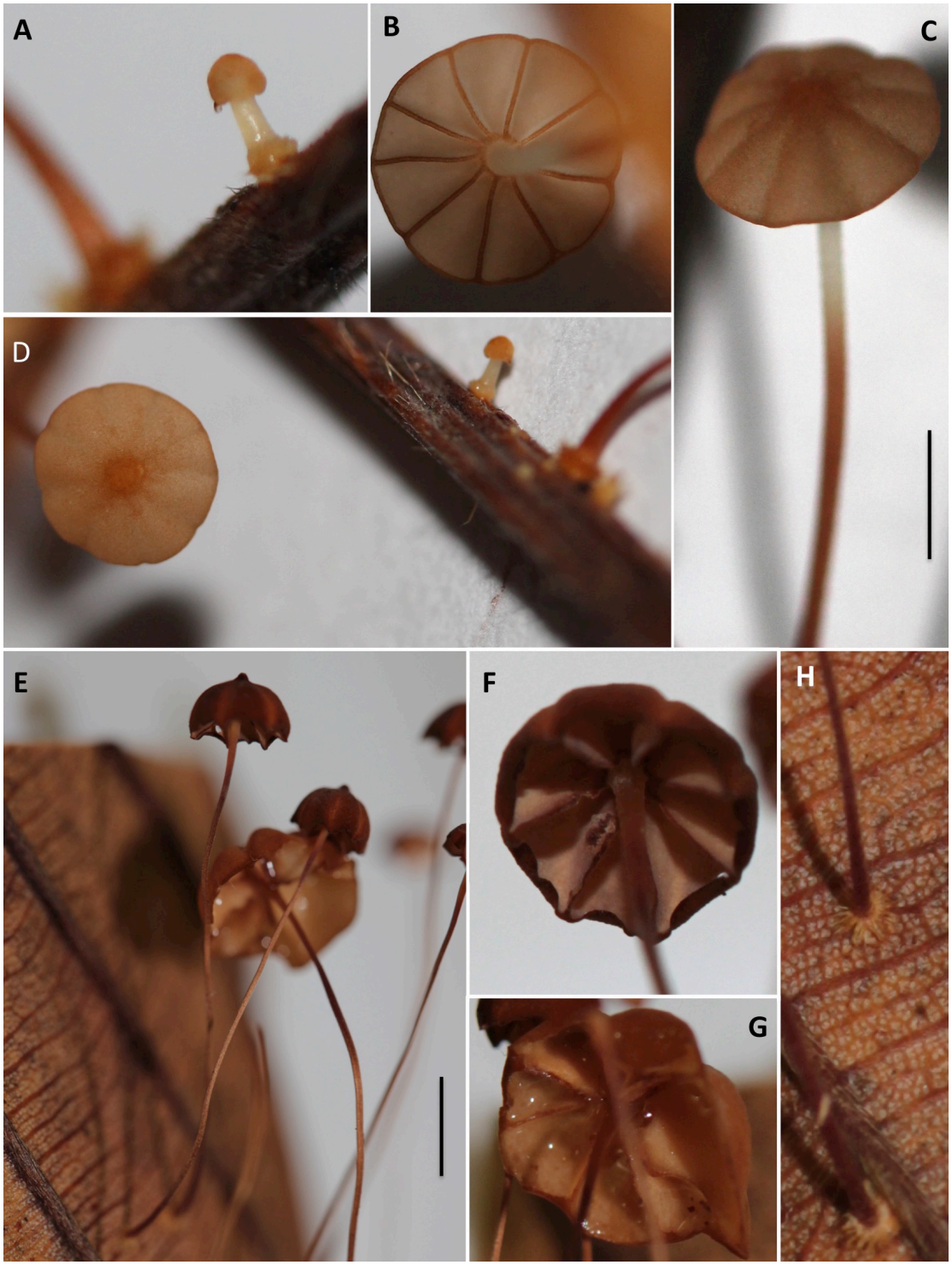


Fig. 11B ***Marasmius*** **"orange5"**

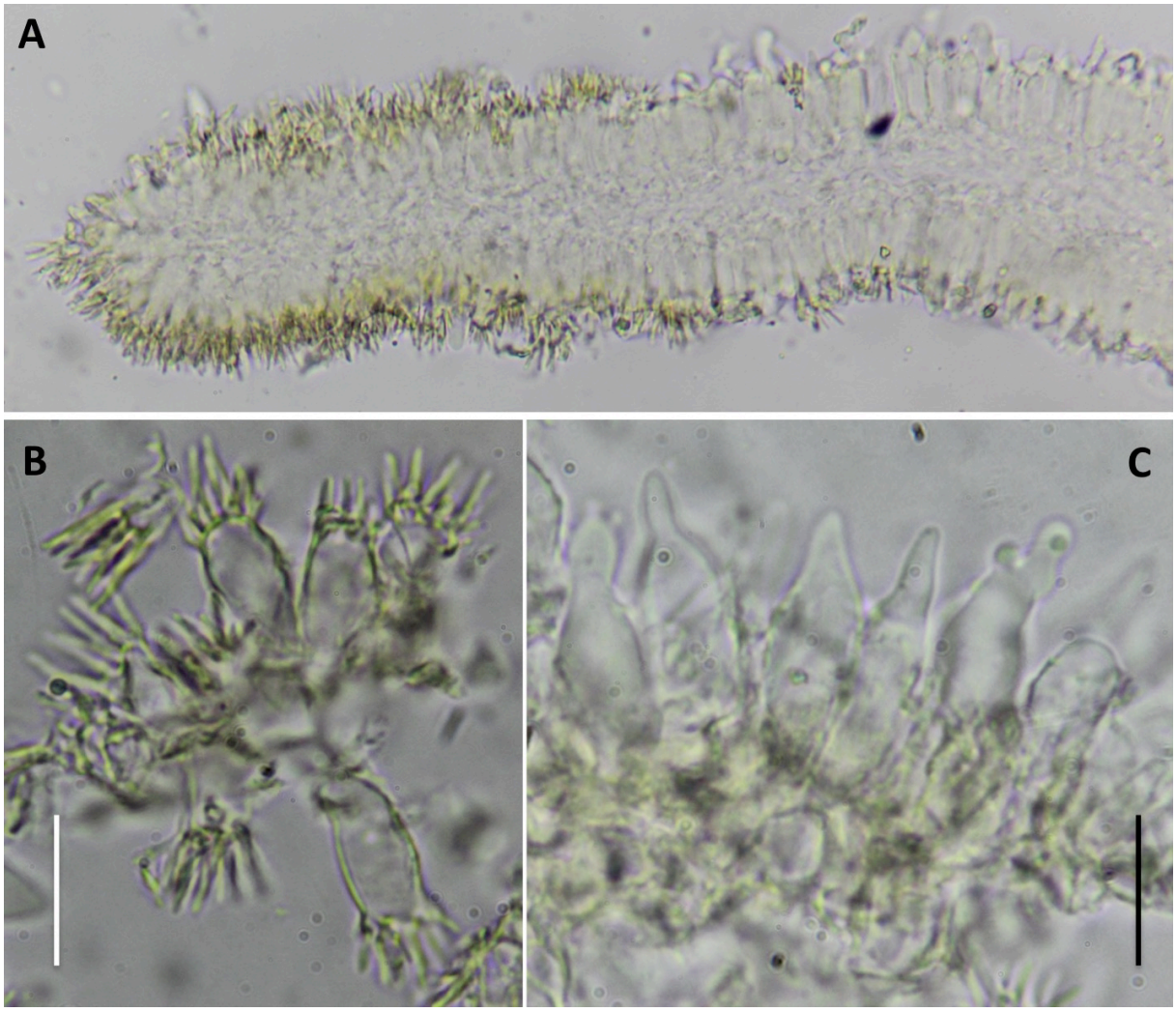


Fig. 12A ***Marasmius haediniformis***

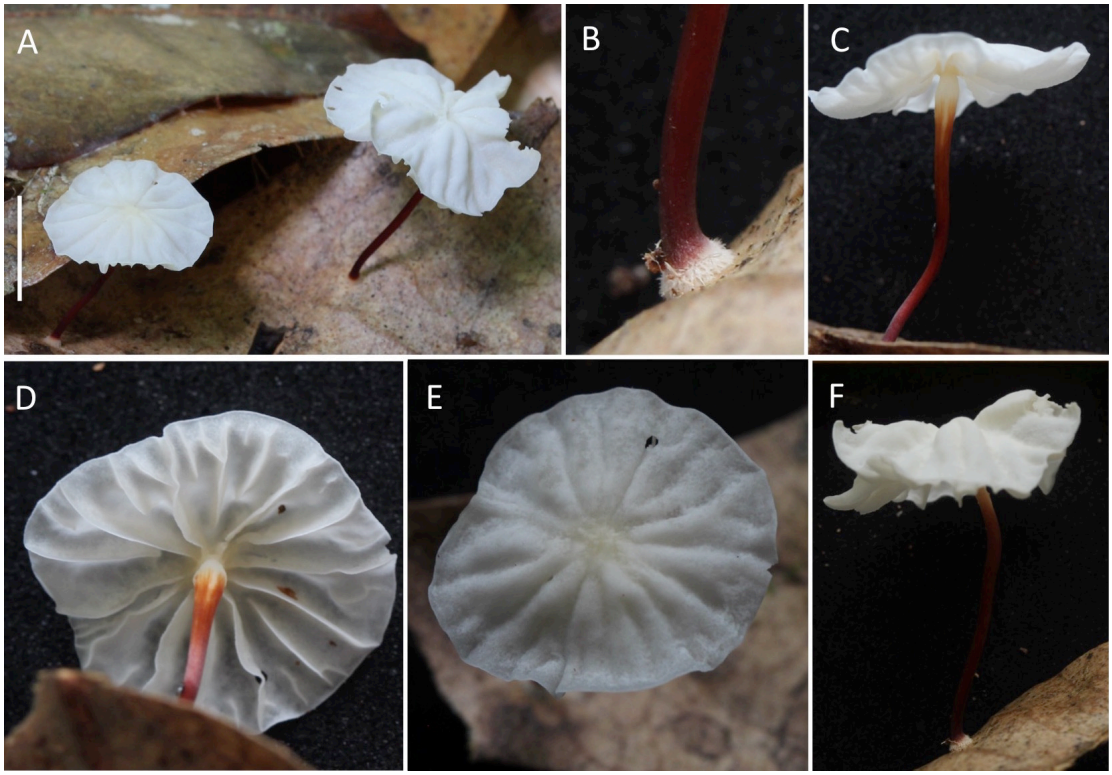


Fig. 12B ***Marasmius haediniformis***

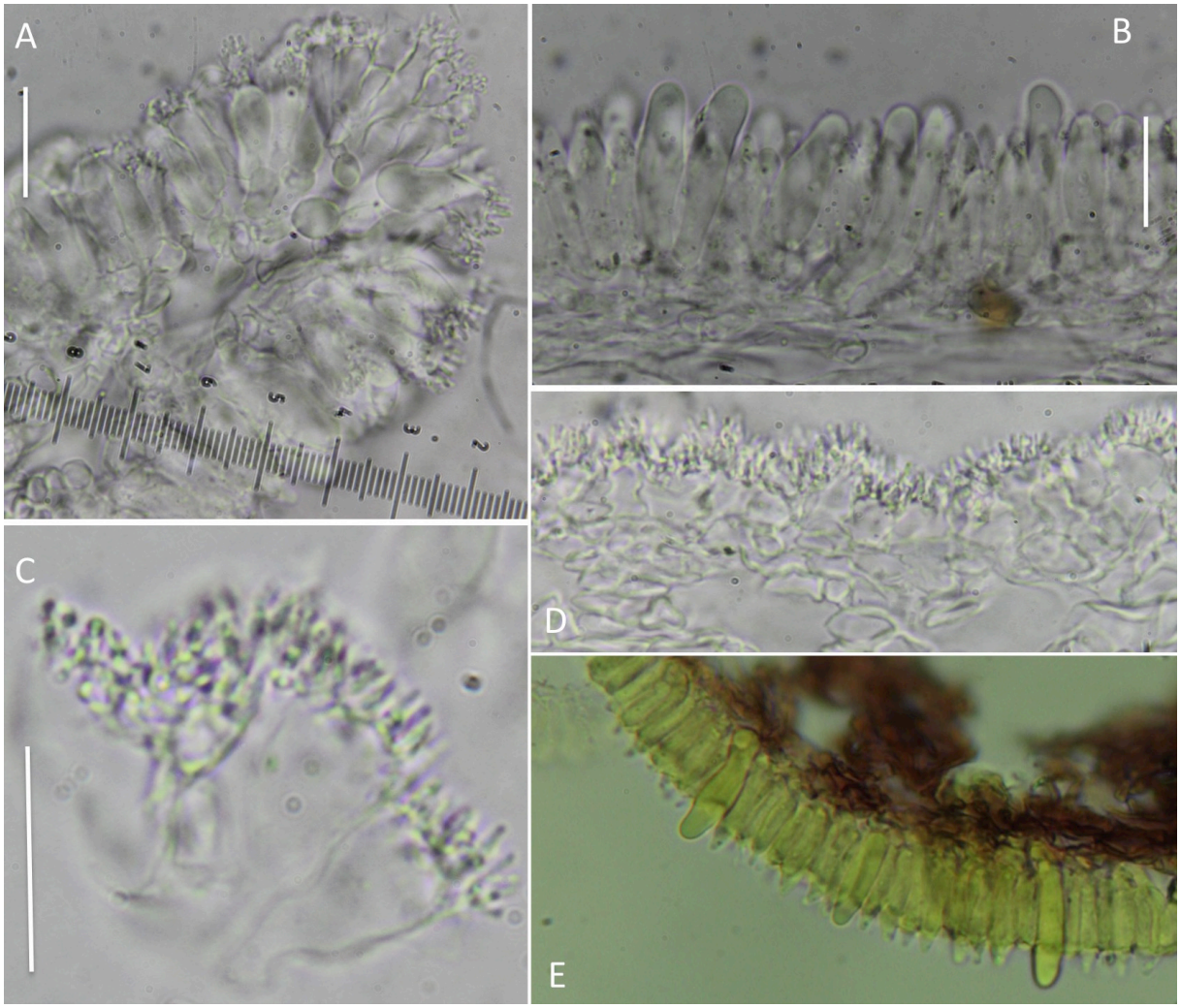


Fig. 13A ***Marasmius congregatus***

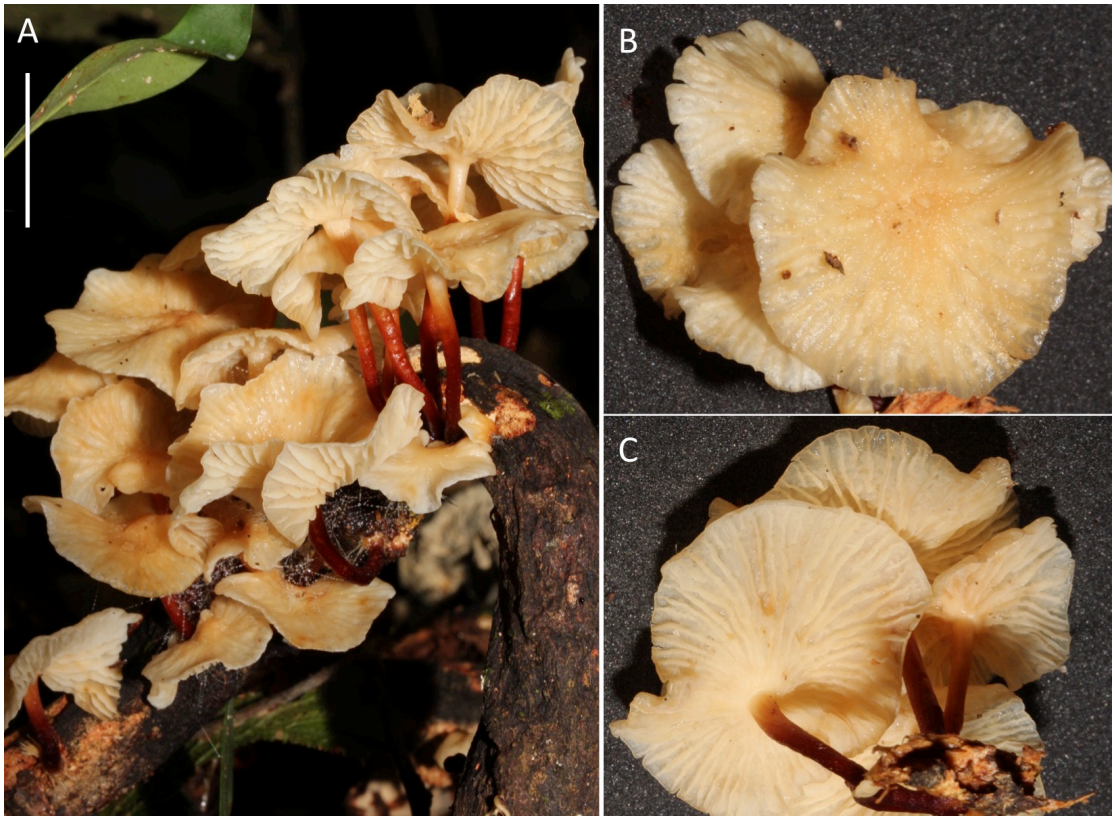


Fig. 13B ***Marasmius congregates***

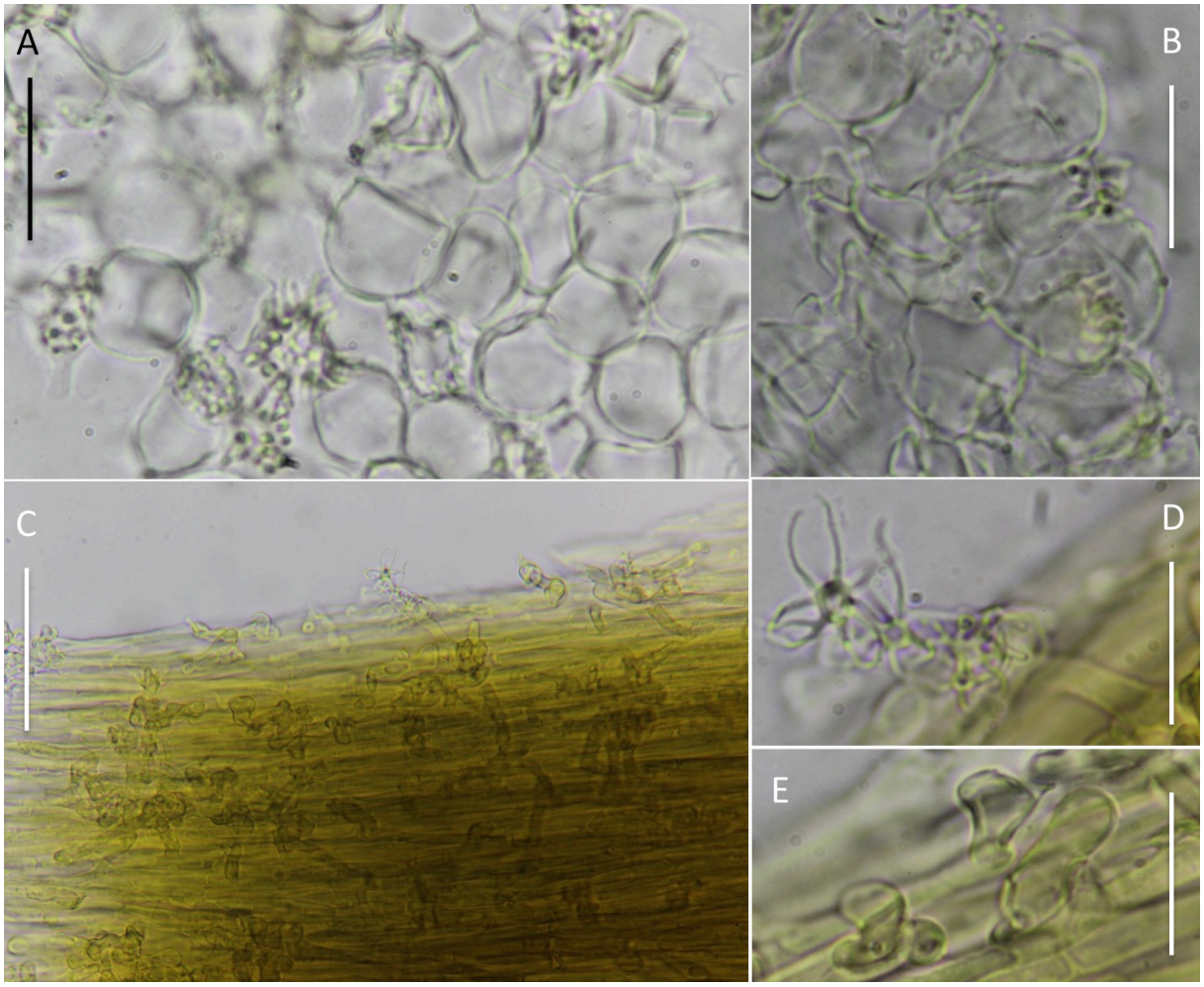


Fig. 14 ***Marasmius*** **"orange10"**

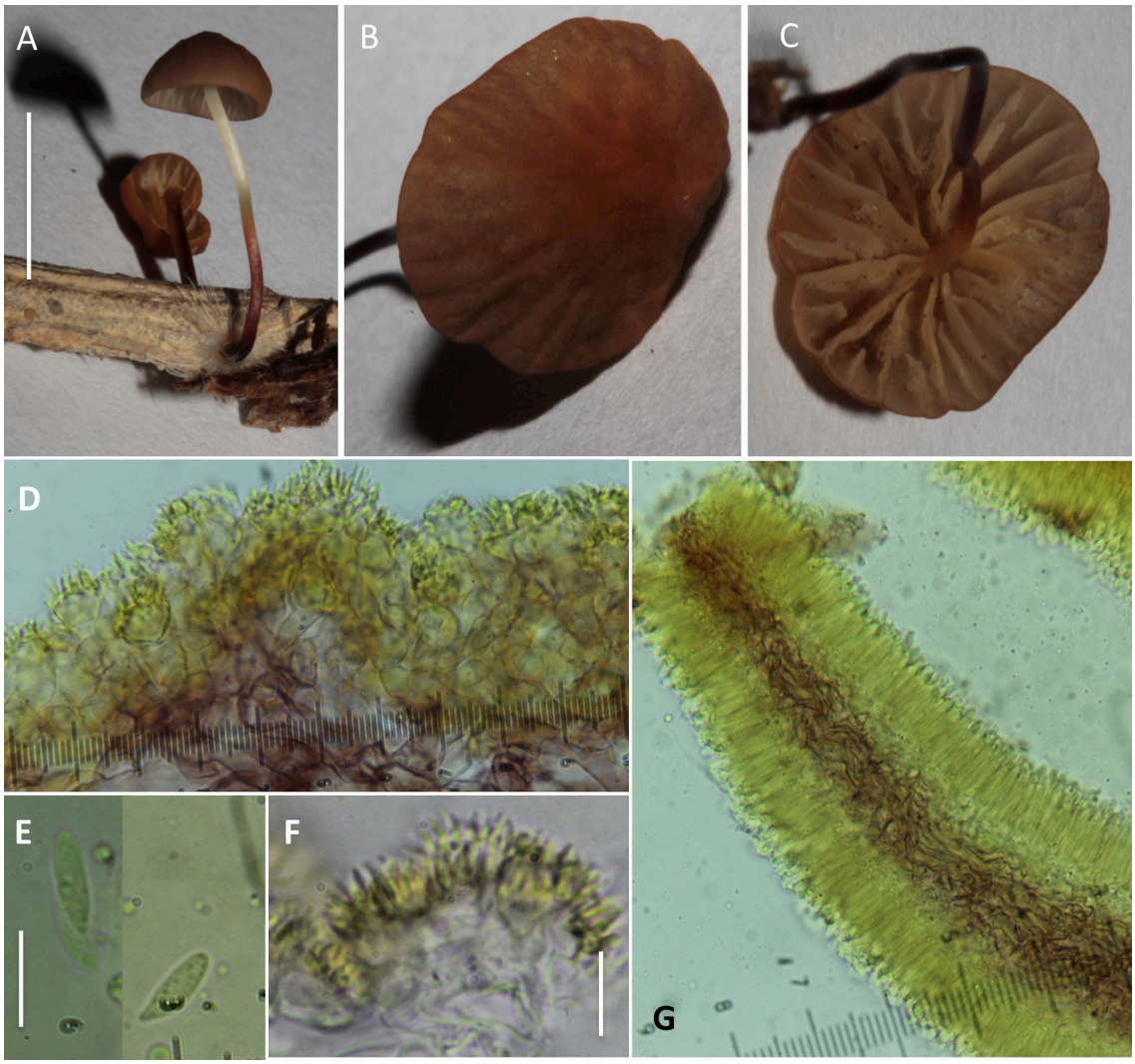


Fig. 15A ***Marasmius bellus***

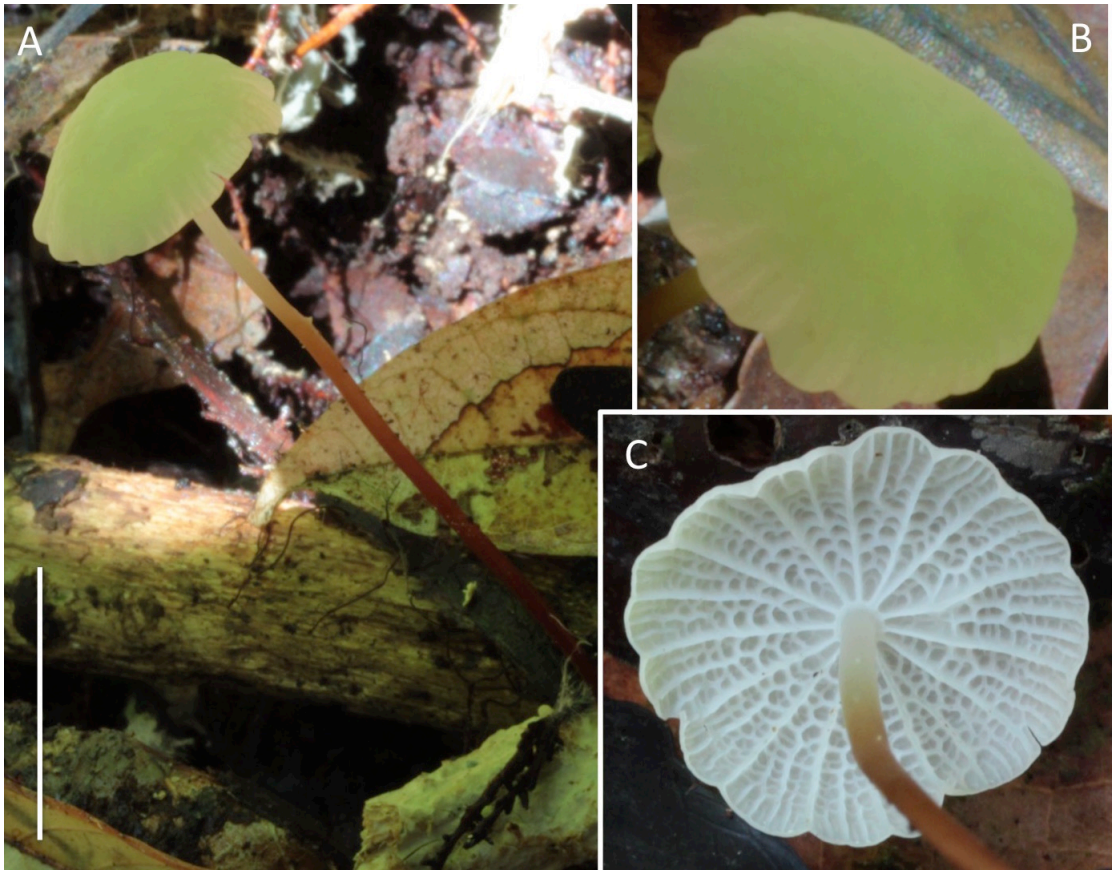


Fig. 15B ***Marasmius bellus***

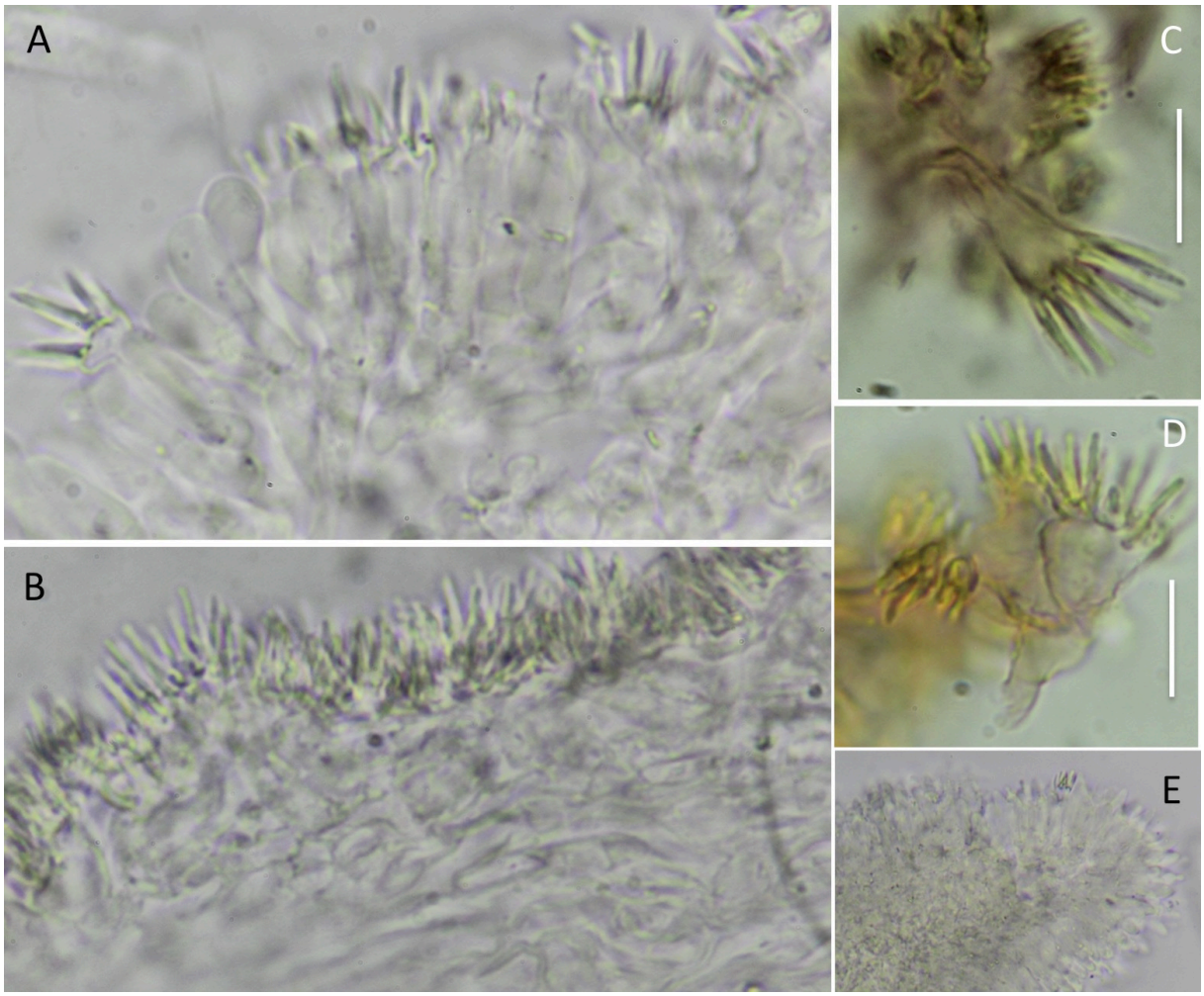


Fig. 16A ***Marasmius*** **"orange2"**

Fig. 16B ***Marasmius*** **"orange2"**

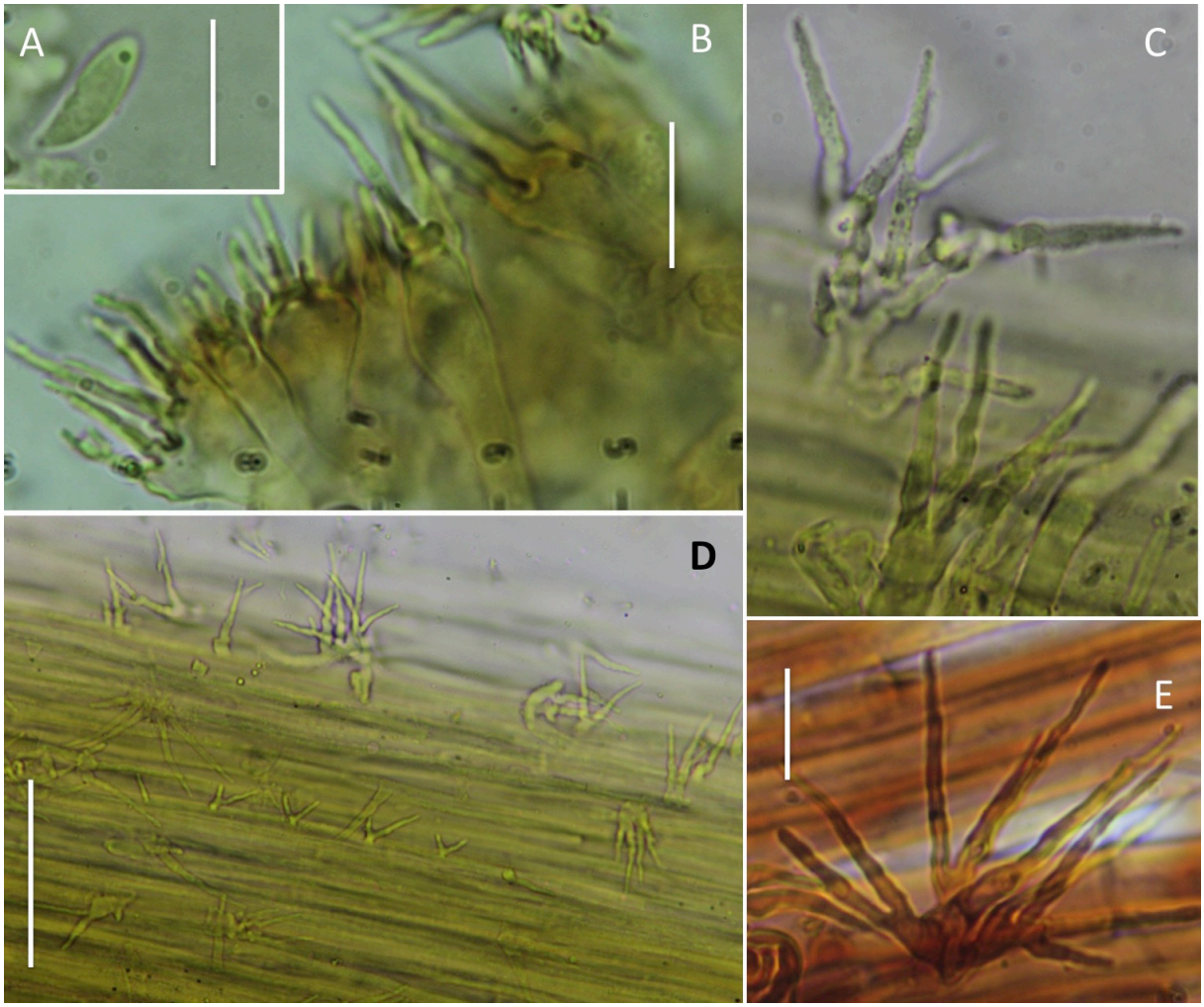


Fig. 17A ***Marasmius ruber***

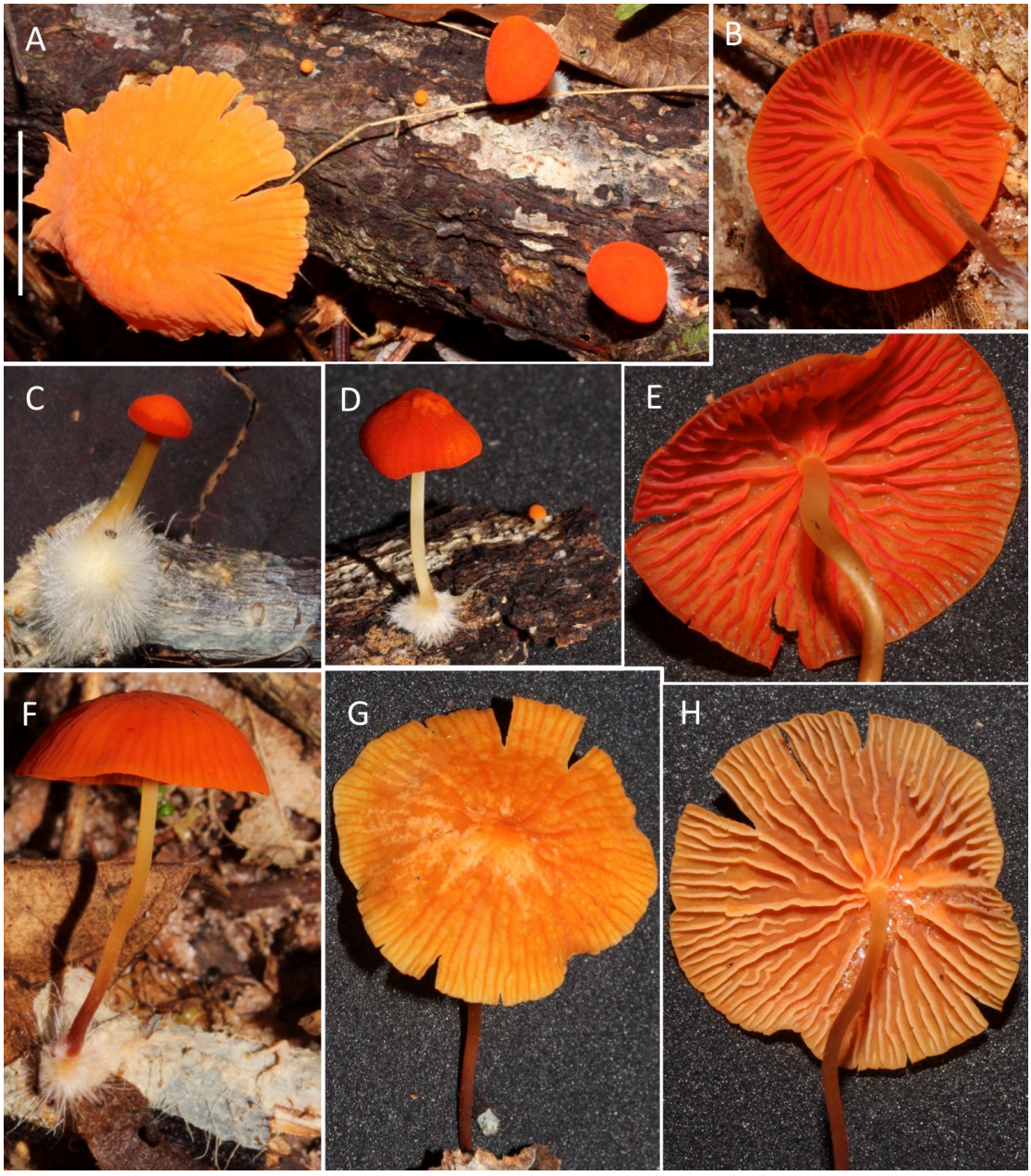


Fig. 17B ***Marasmius ruber***

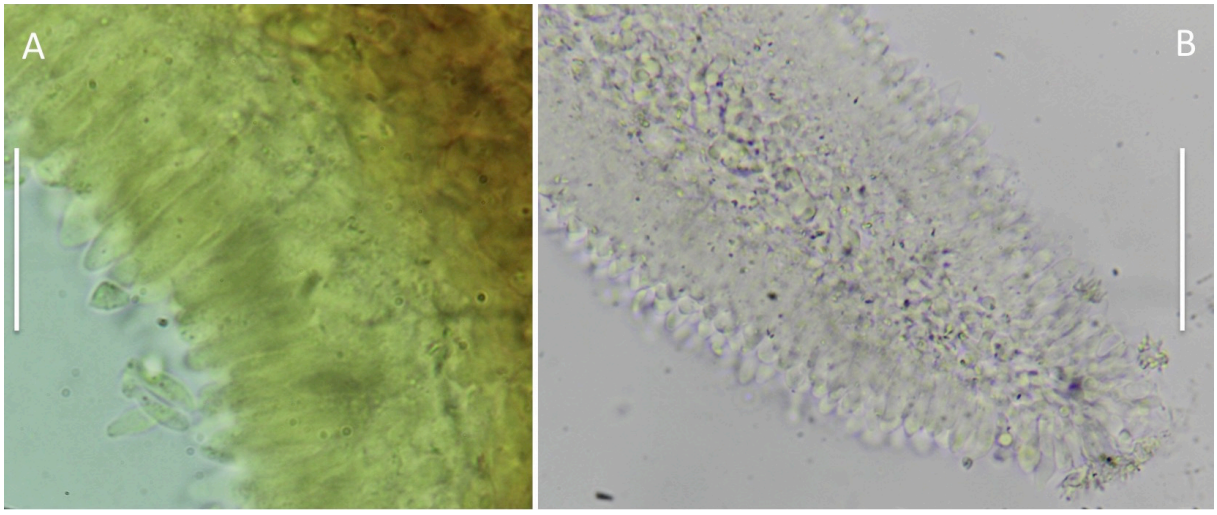


Fig. 18A ***Marasmius*** **"orange4"**

Fig. 18B ***Marasmius*** **"orange4"**

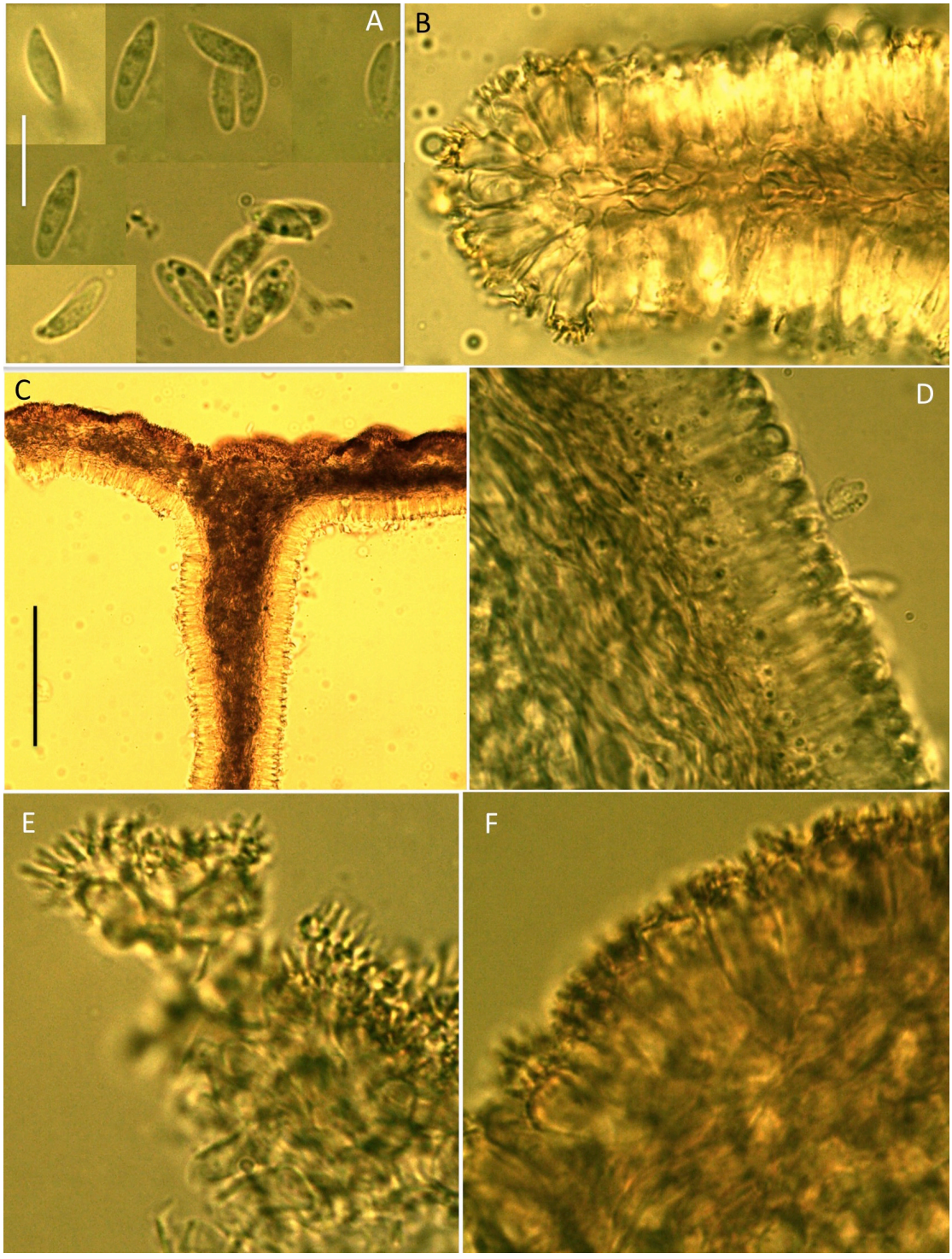


Fig. 19A ***Marasmius cladophyllus***

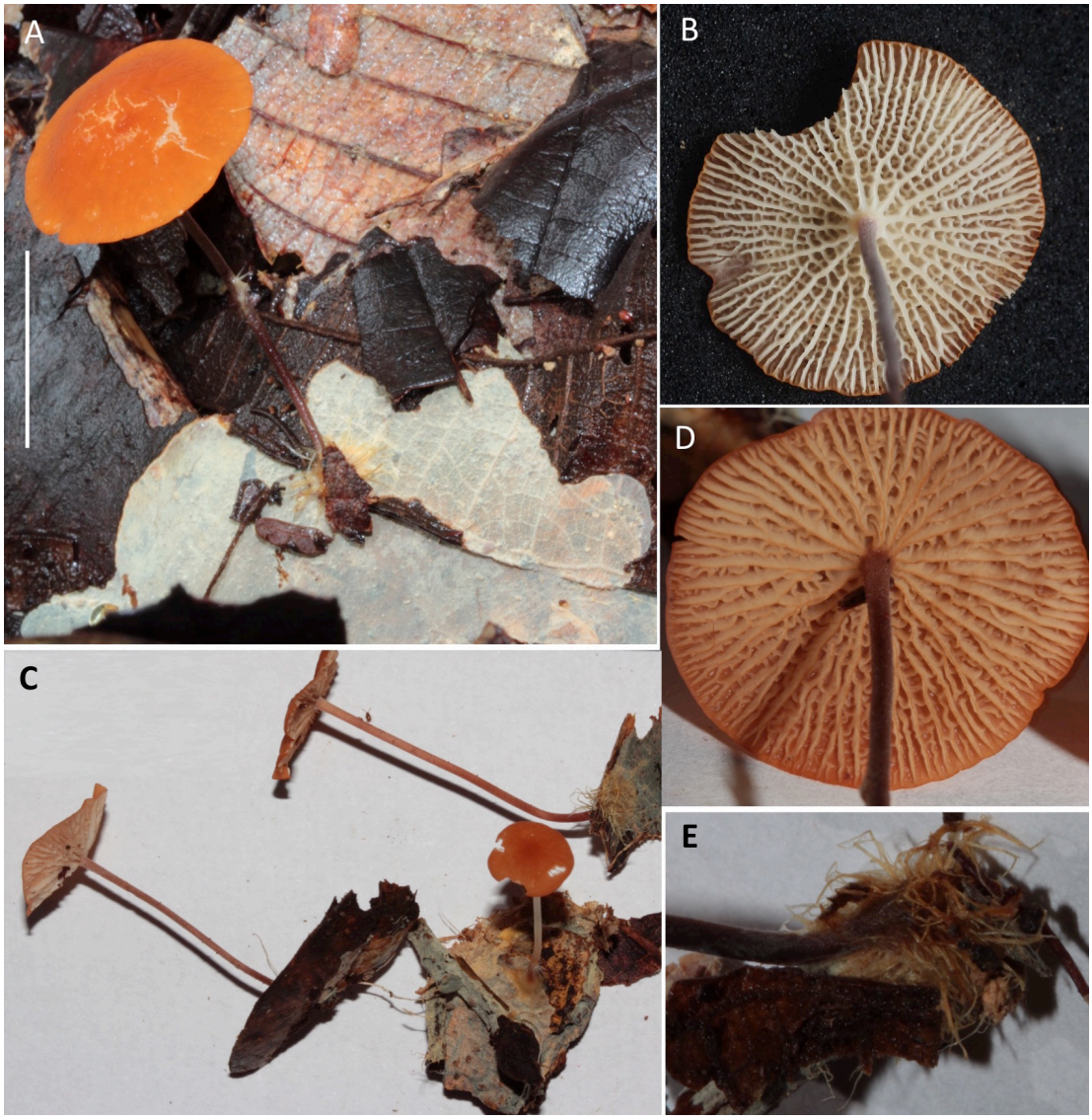


Fig. 19B ***Marasmius cladophyllus***

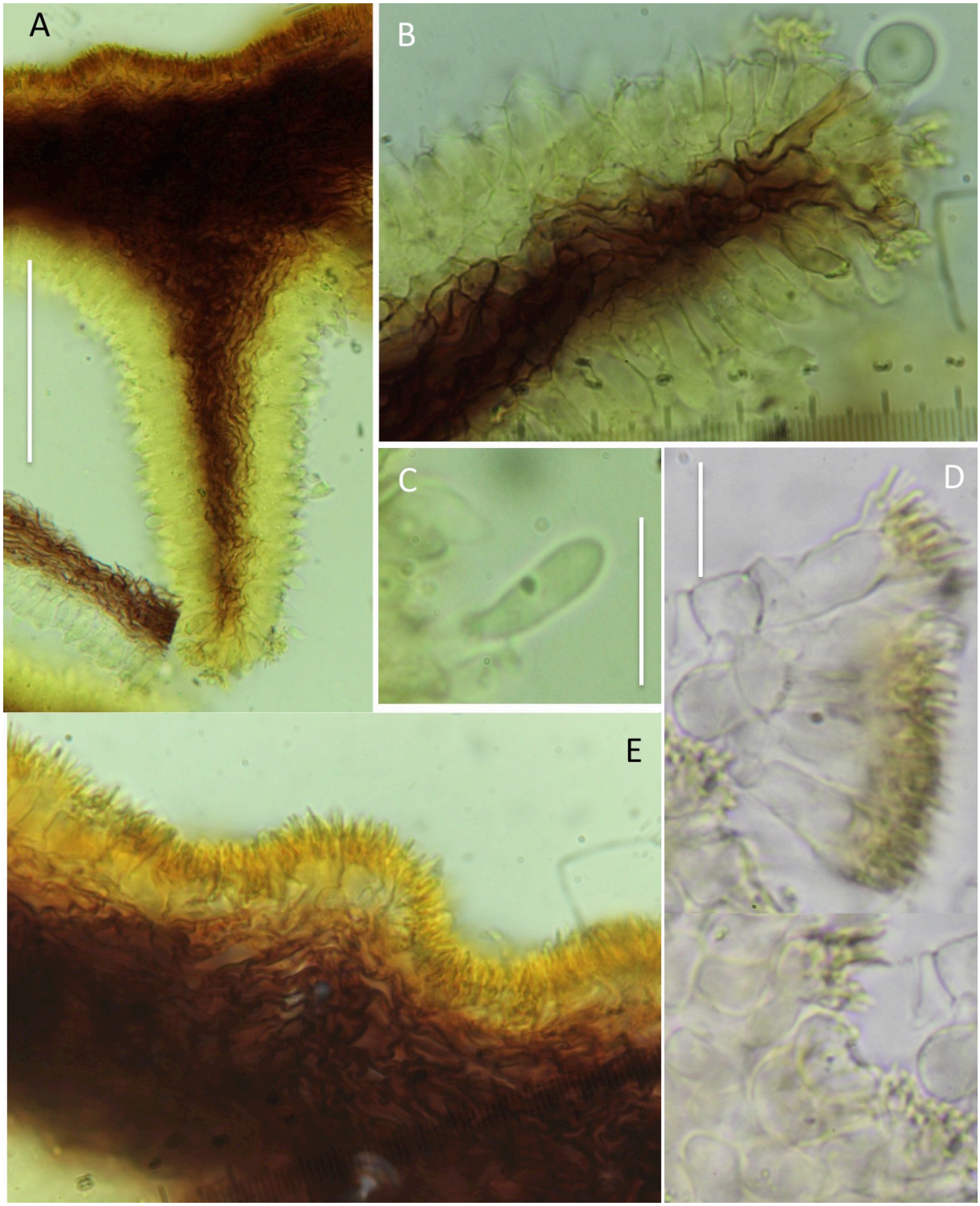


Fig. 20A ***Marasmius*** **cf.** ***digilioi***

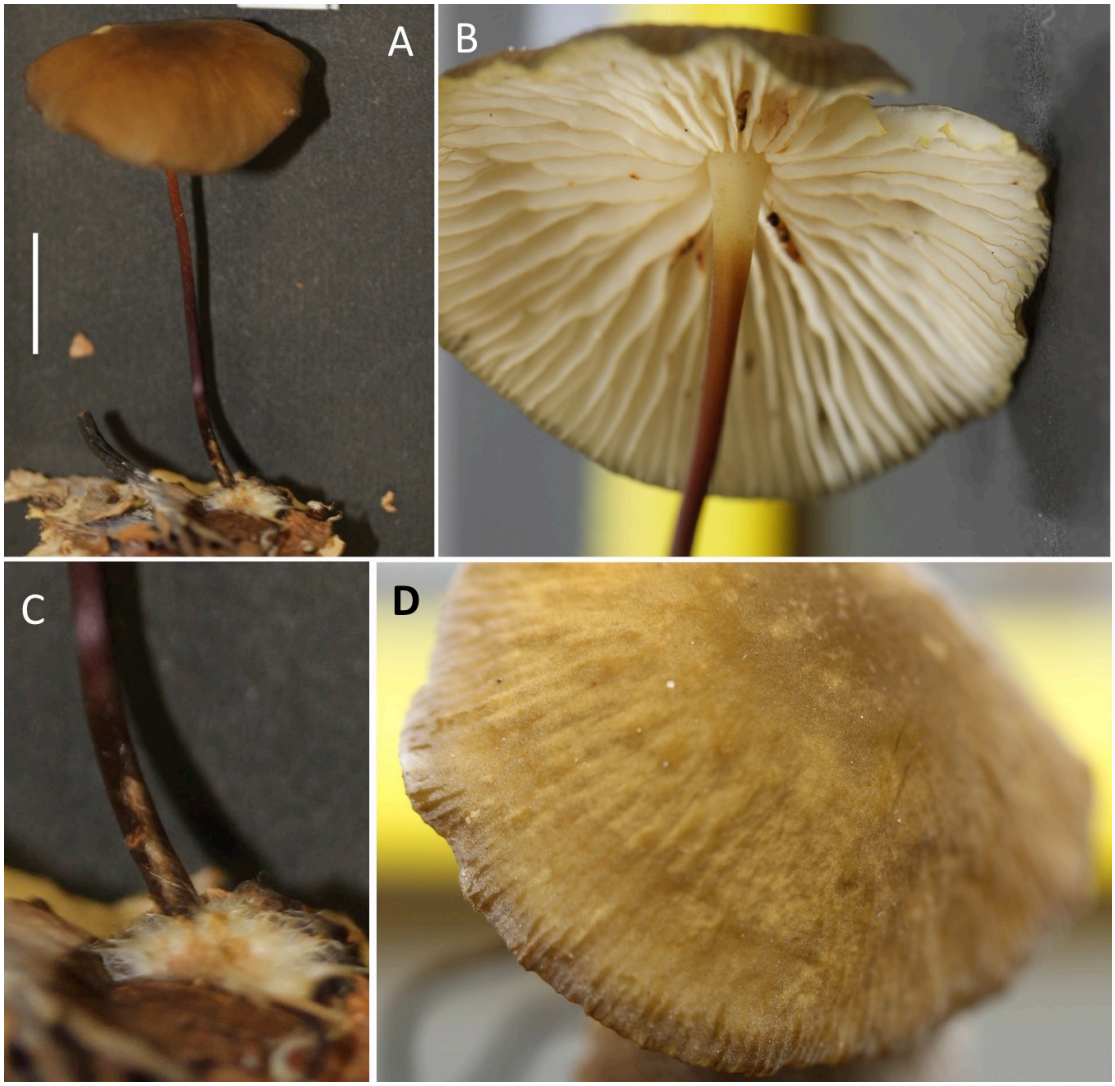


Fig. 20B ***Marasmius*** **cf.** ***digilioi***

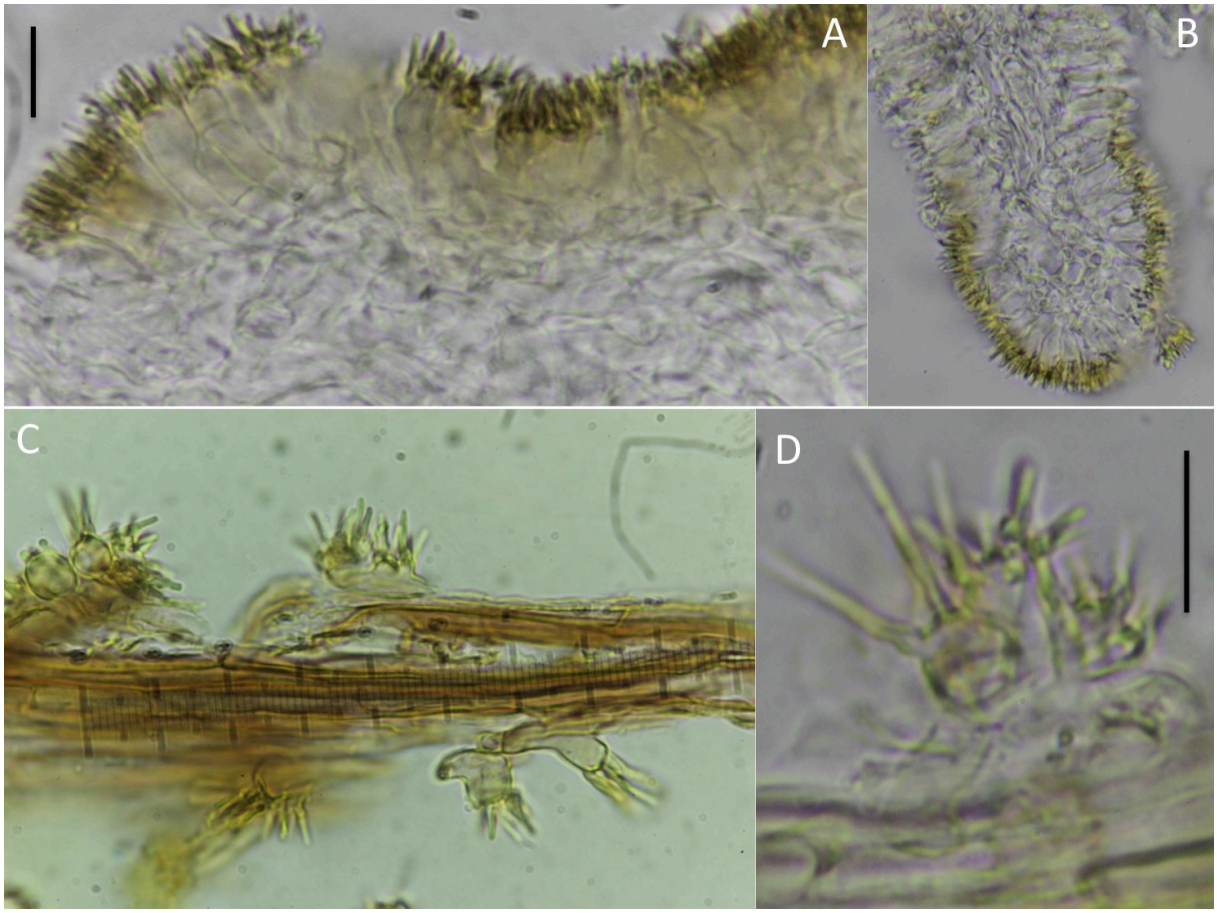


Fig. 21A ***Marasmius*** **cf.** ***trinitatis***

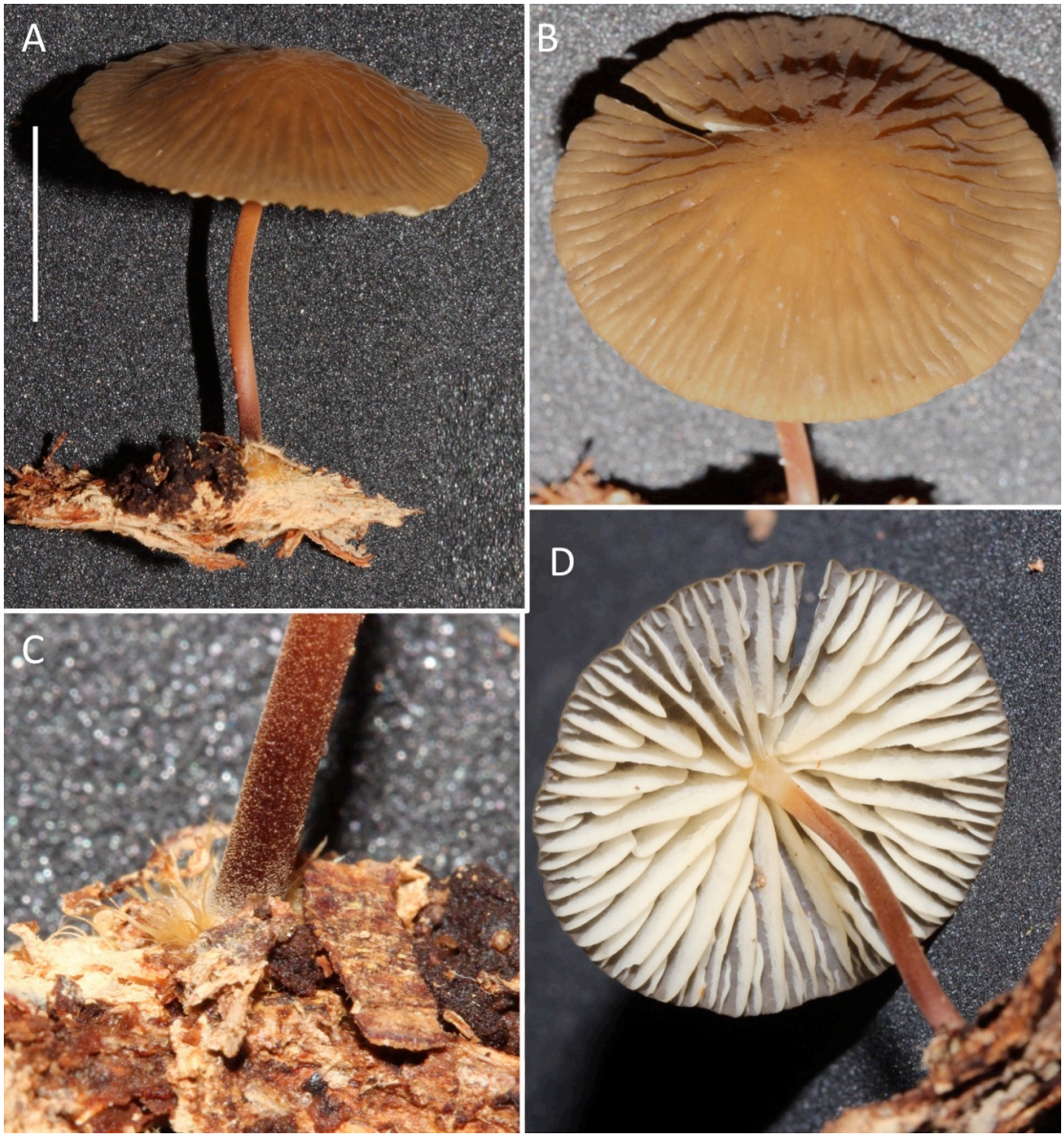


Fig. 21B ***Marasmius*** **cf.** ***trinitatis***

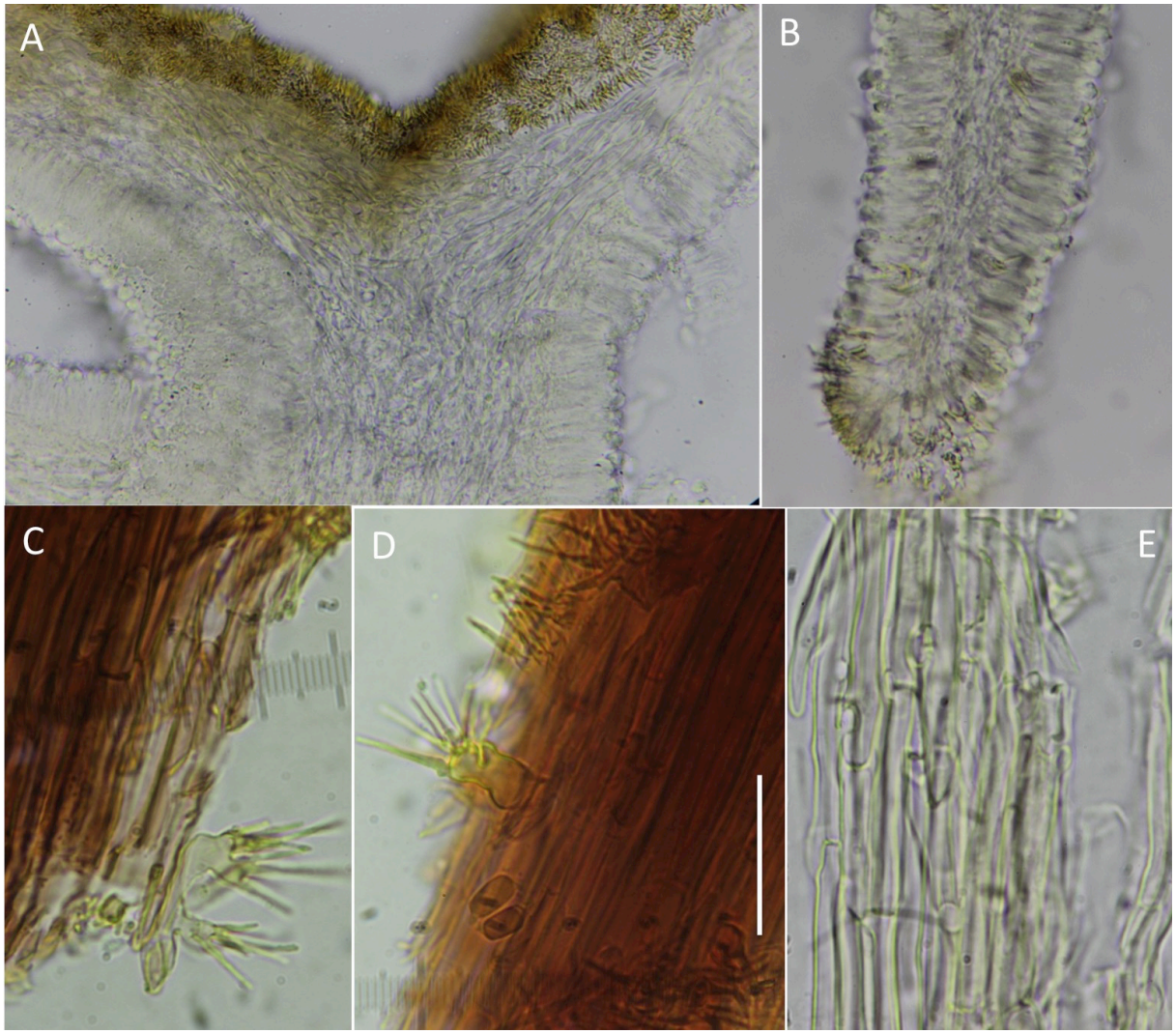


Fig. 22A ***Marasmius phaeus***

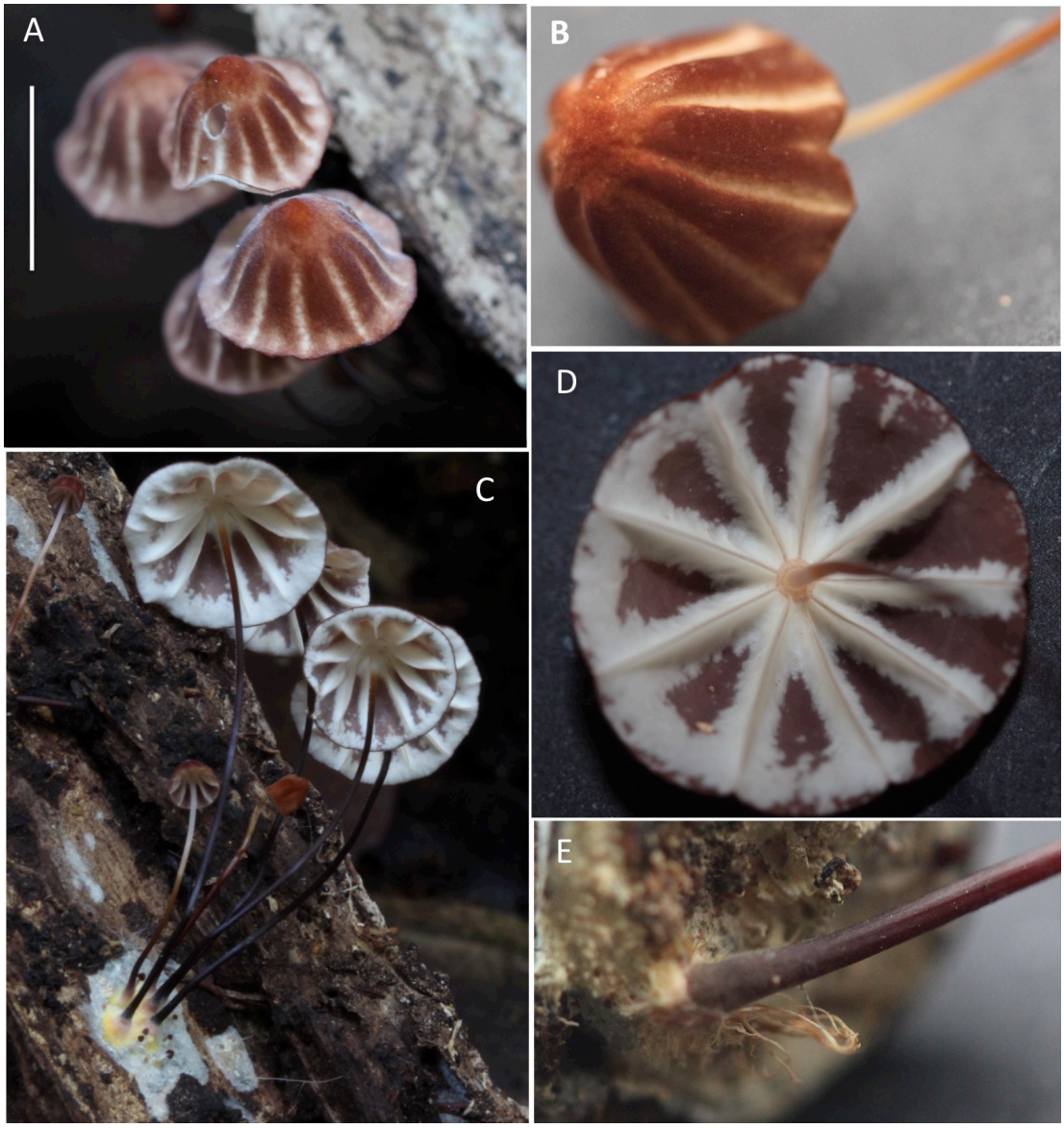


Fig. 22B ***Marasmius phaeus***

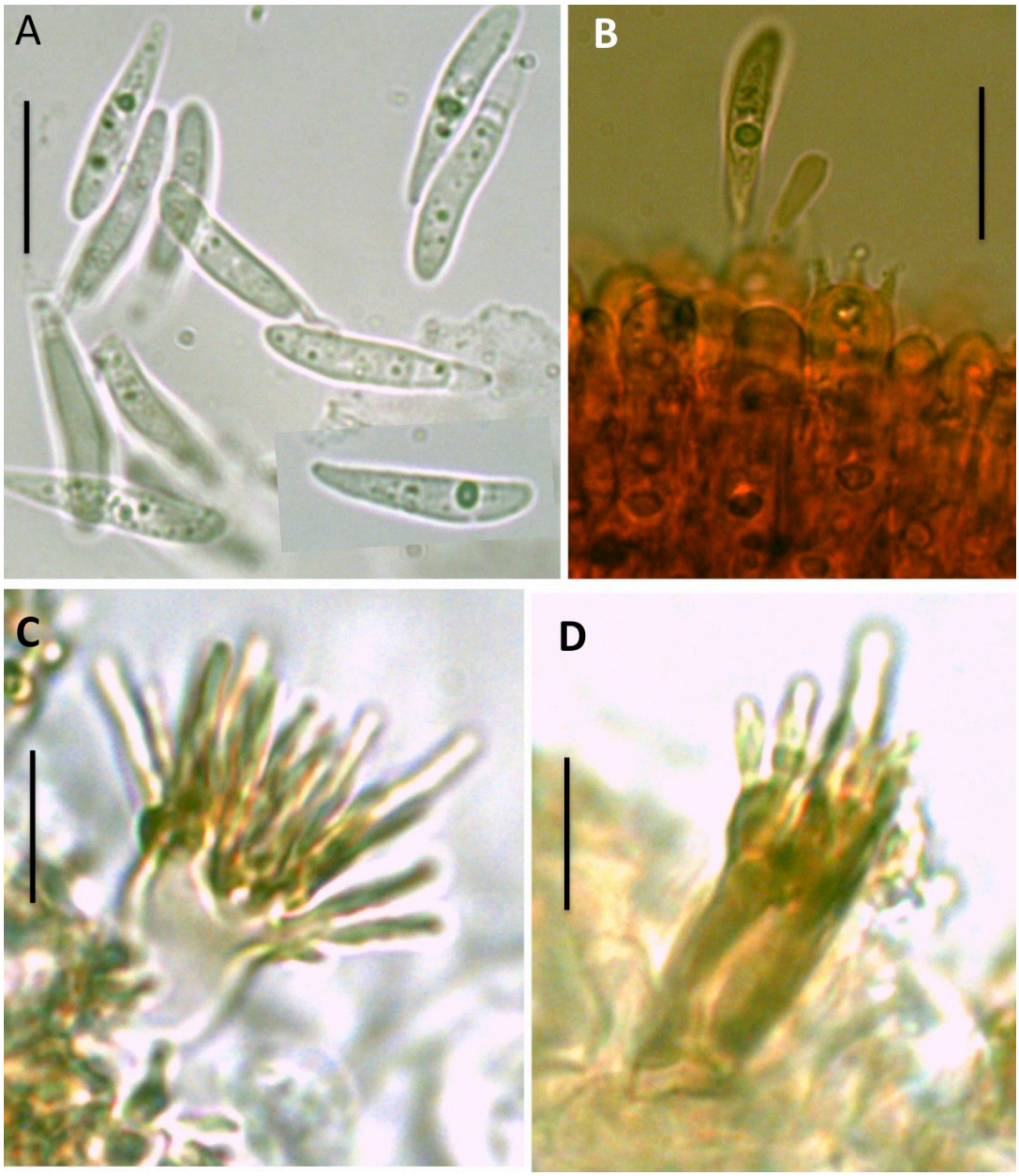


Fig. 23A ***Marasmius hypophaeus***

Fig. 23B ***Marasmius hypophaeus***

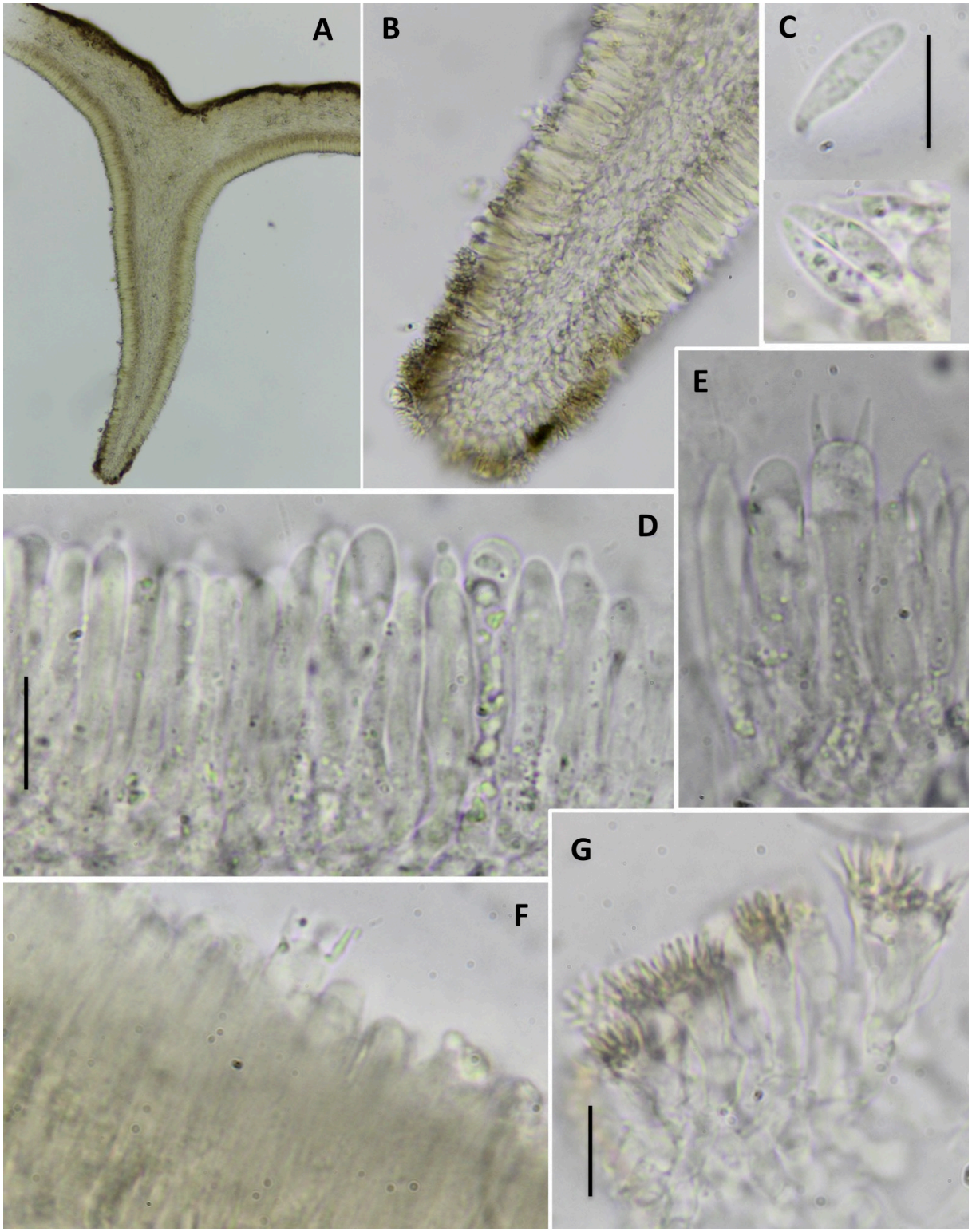


Fig. 24A ***Marasmius haematocephalus***

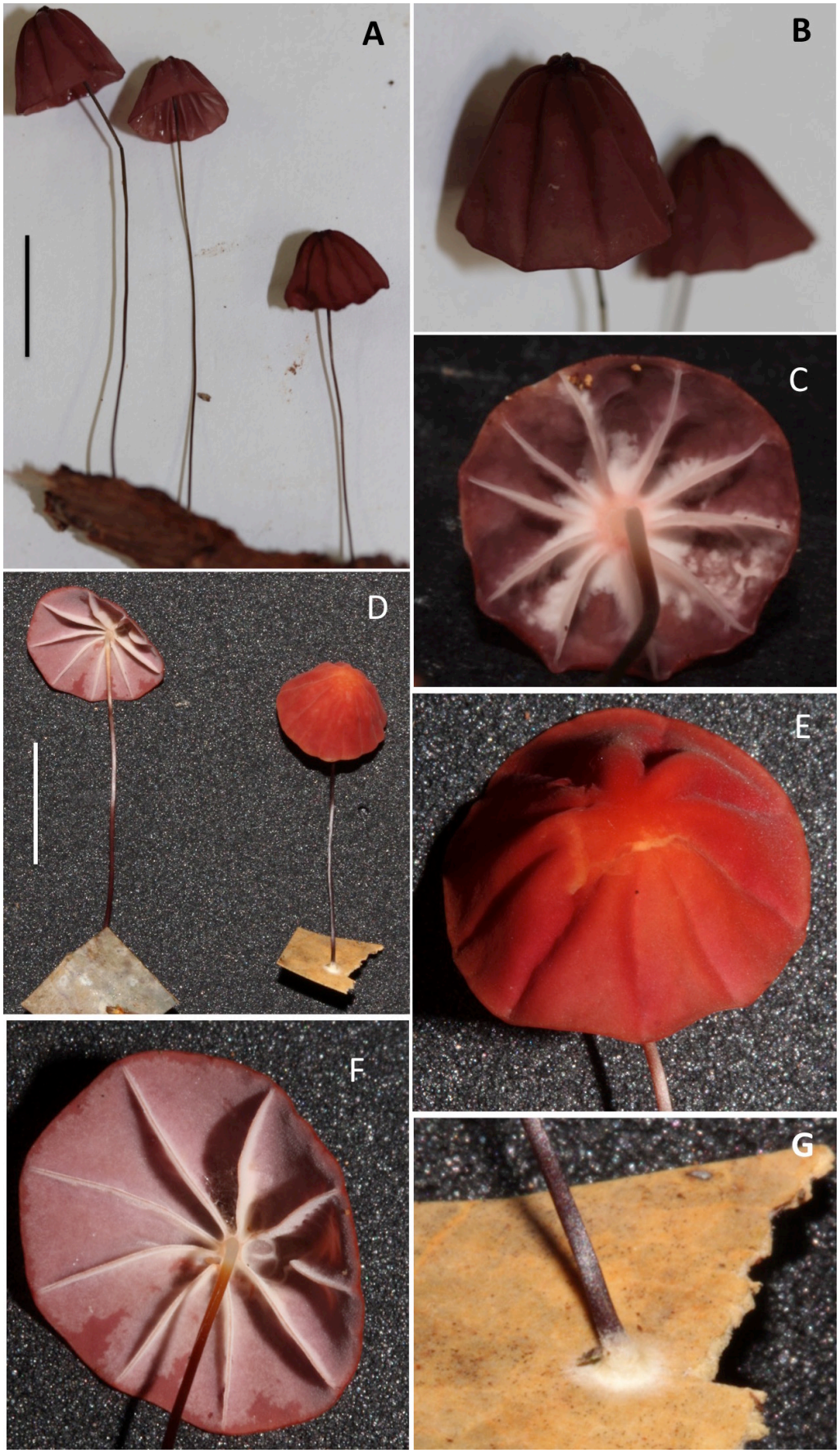


Fig. 24B ***Marasmius haematocephalus***

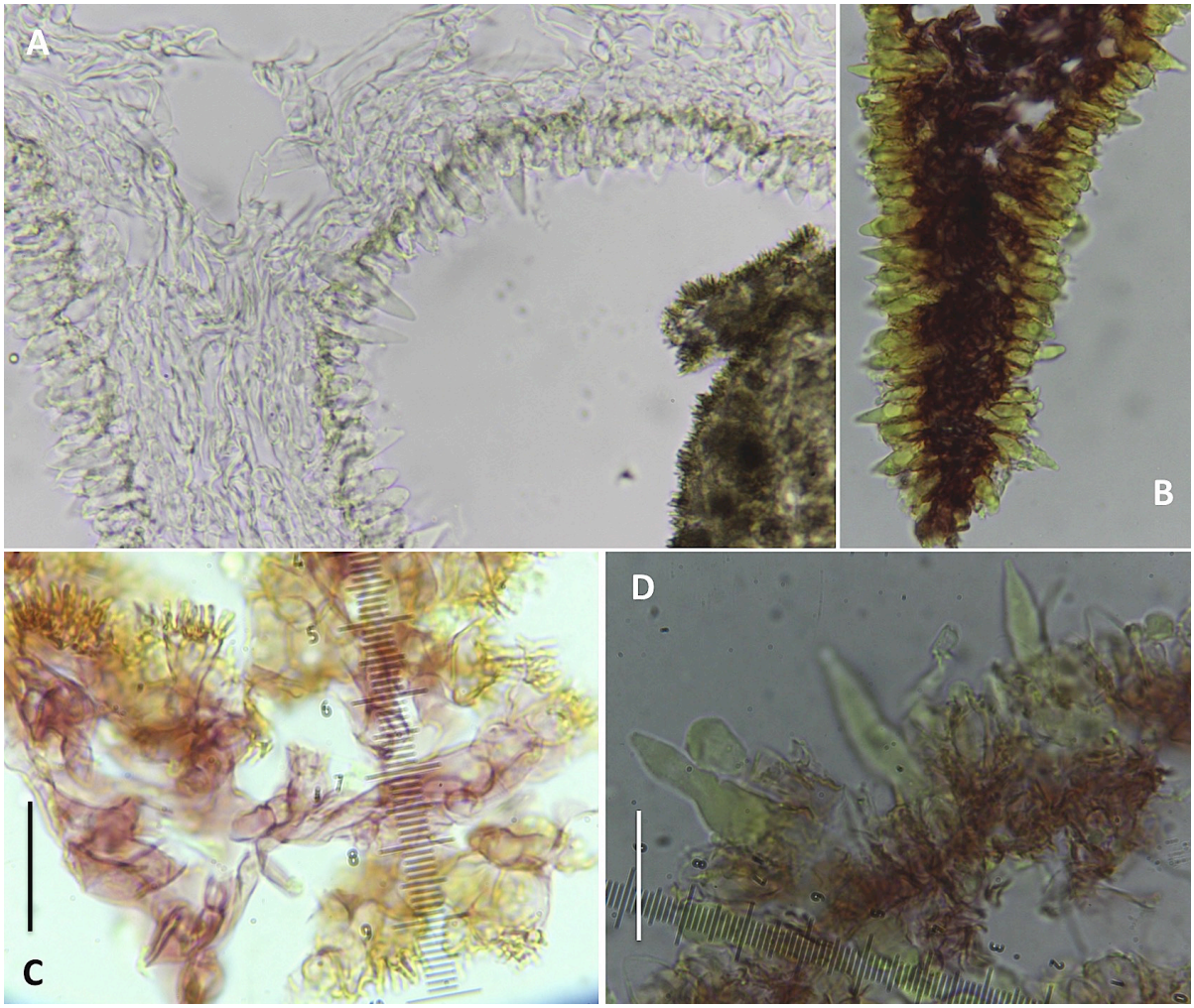


Fig. 25A ***Marasmius berteroi***

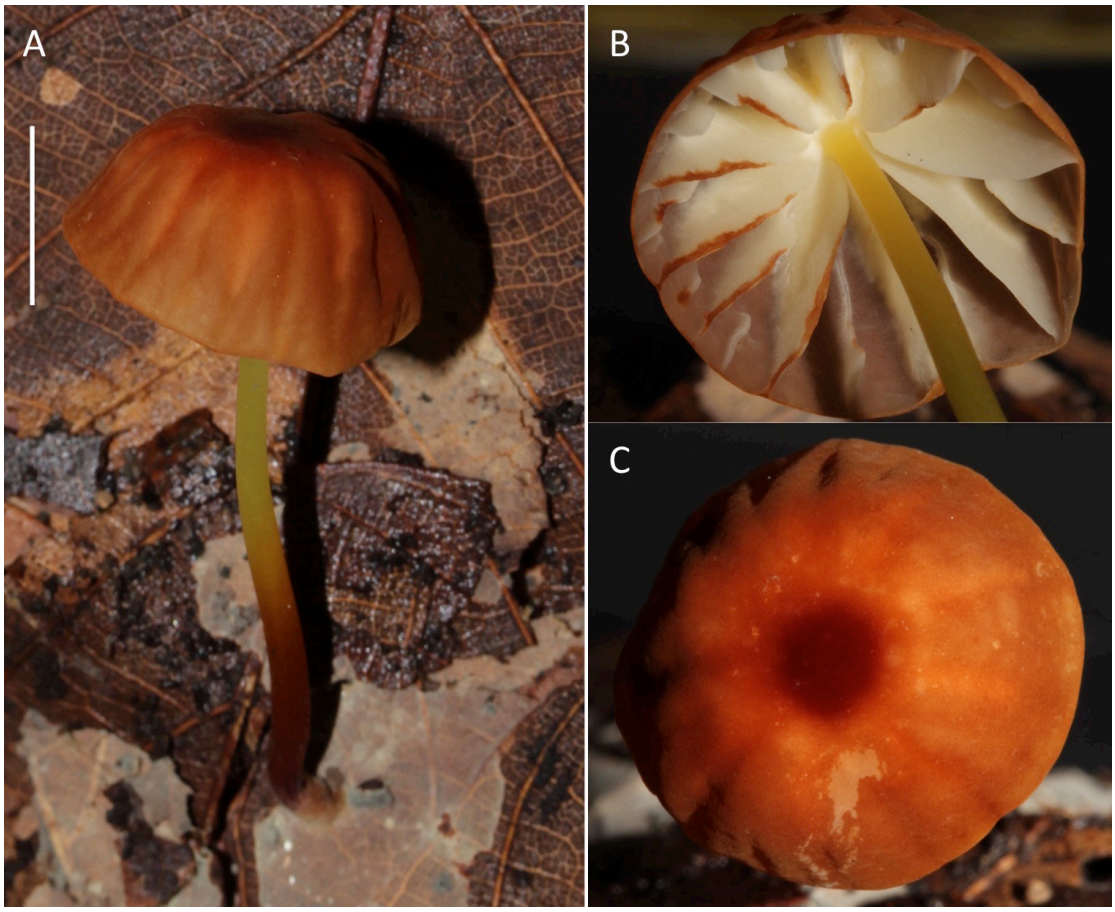


Fig. 25B ***Marasmius berteroi***

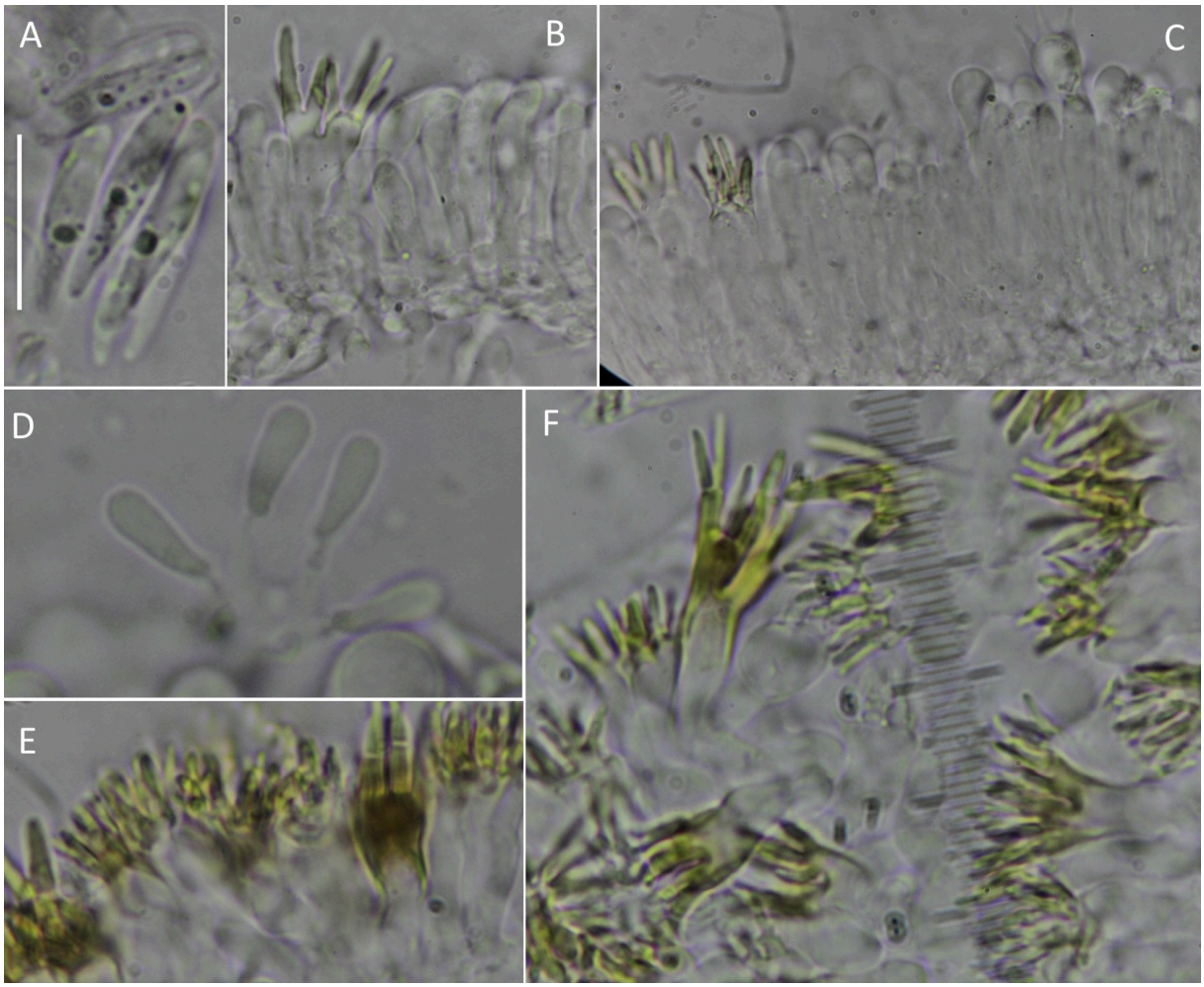


Fig. 26 ***Marasmius*** **"orange7"**

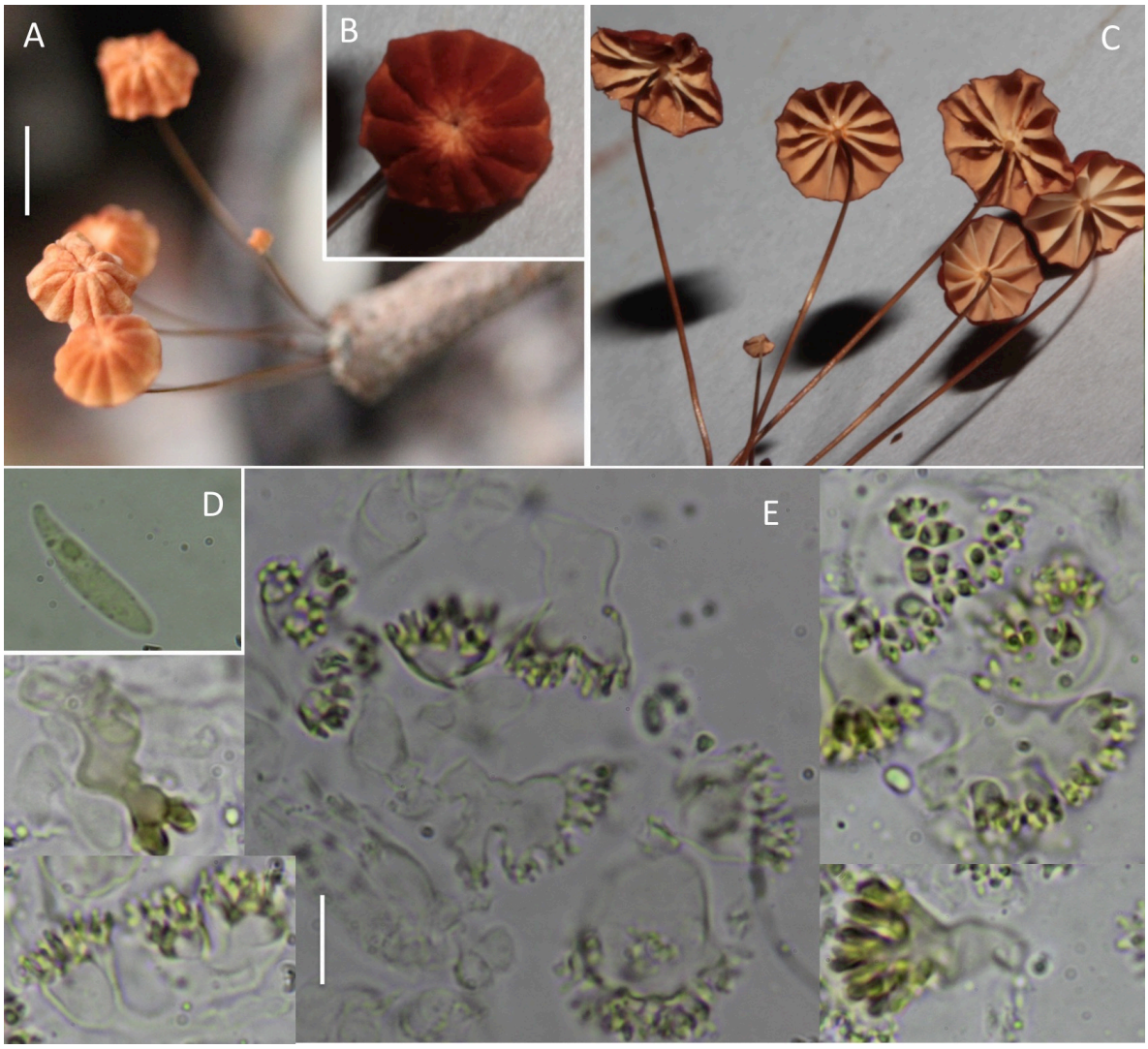


Fig. 27A ***Marasmius*** **cf.** ***guyanensis***

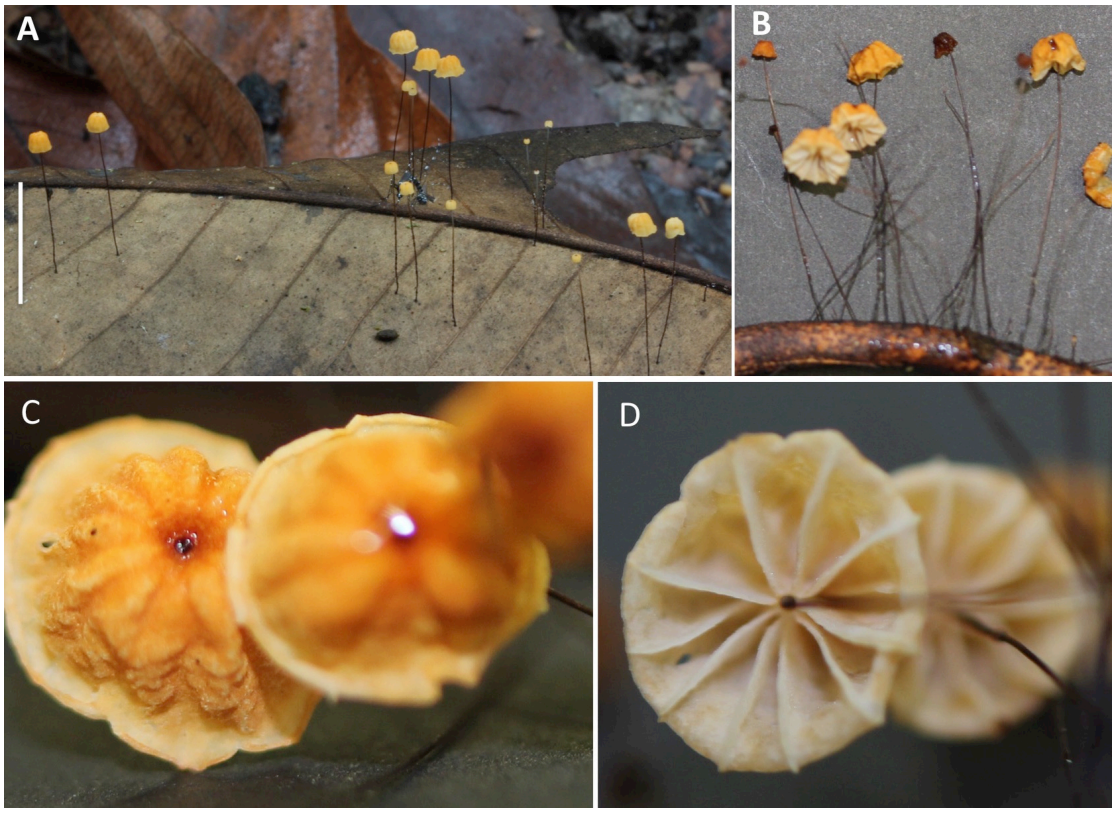


Fig. 27B ***Marasmius*** **cf.** ***guyanensis***

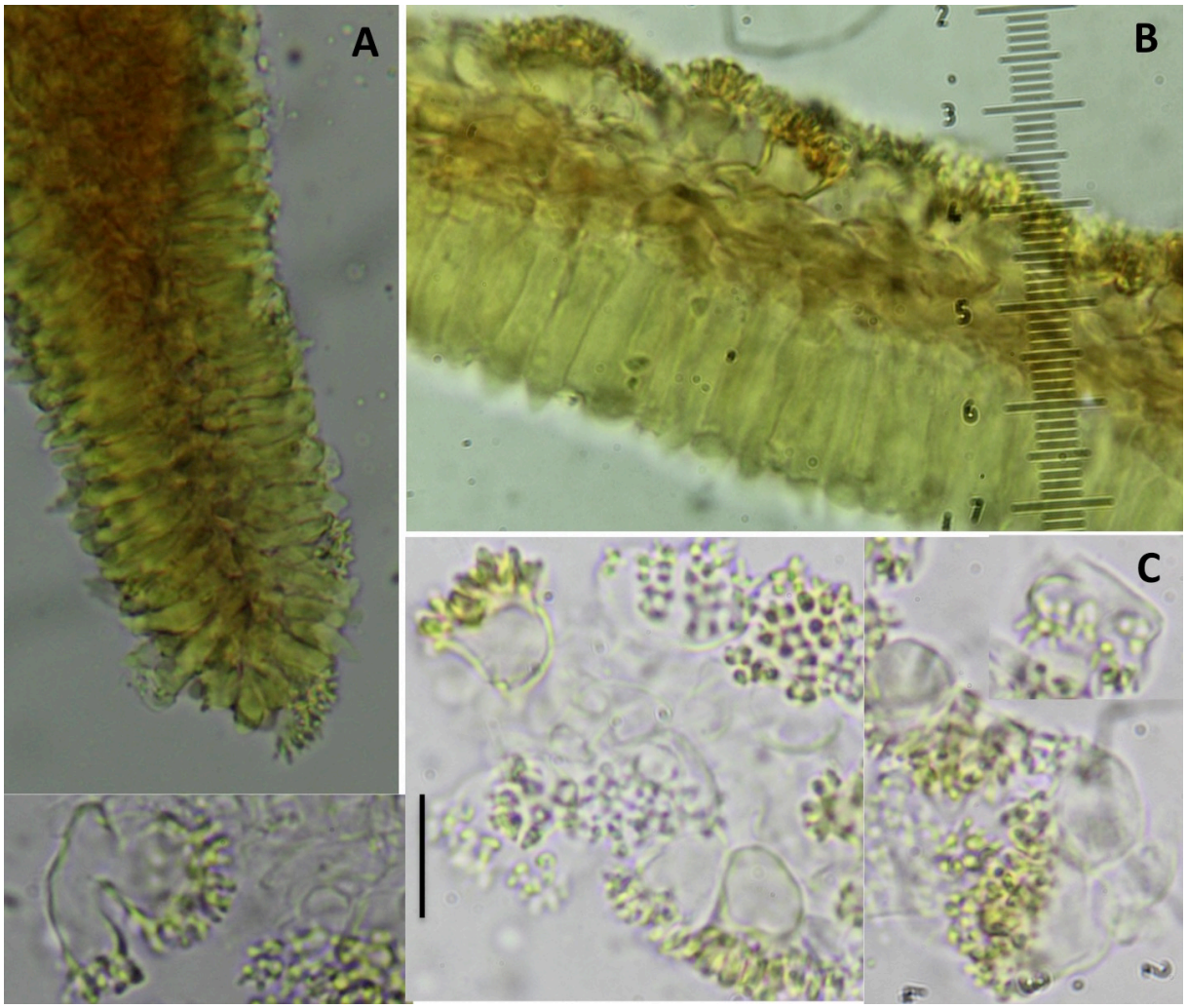


Fig. 28A ***Marasmius tageticolor***

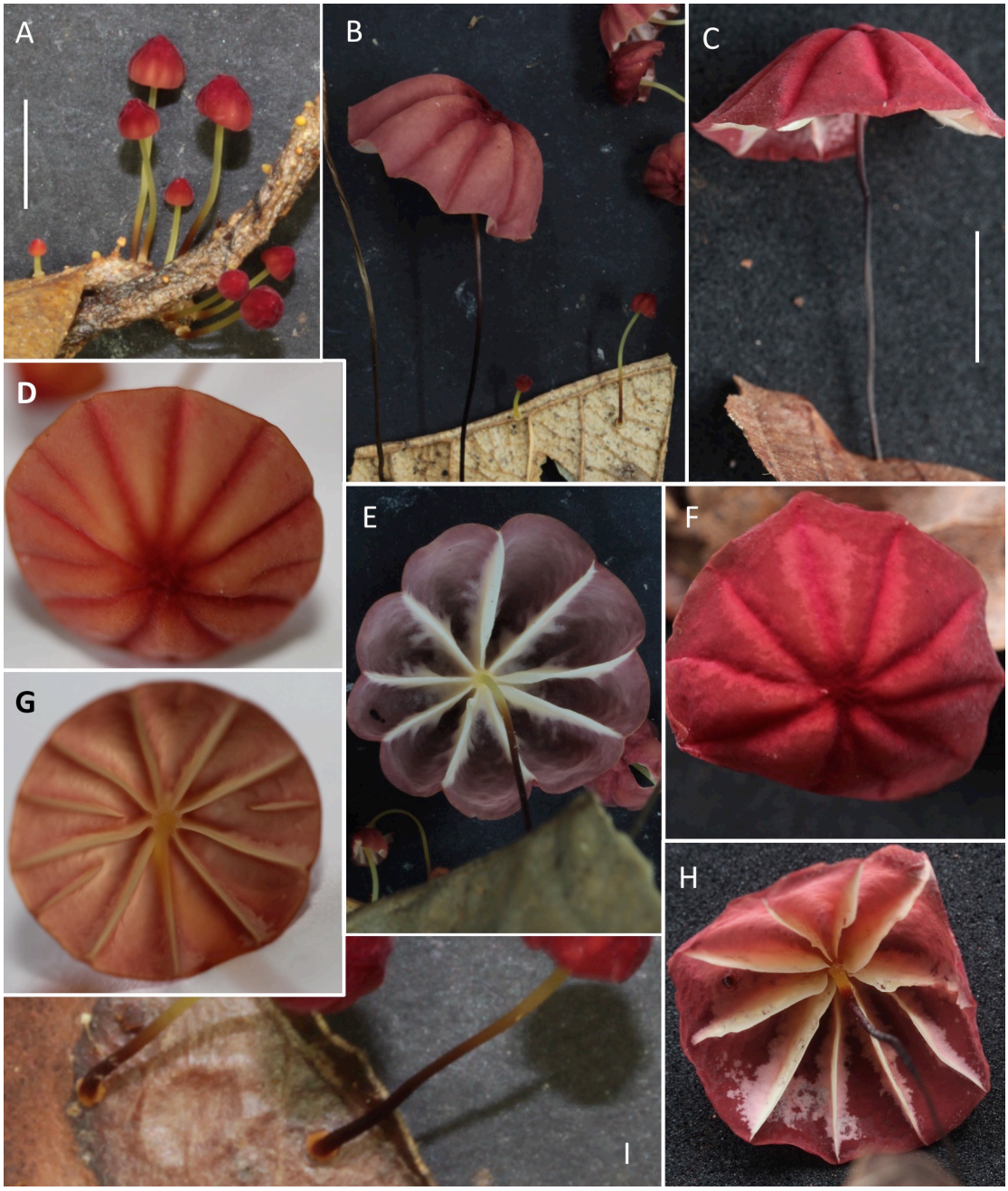


Fig. 28B ***Marasmius tageticolor***

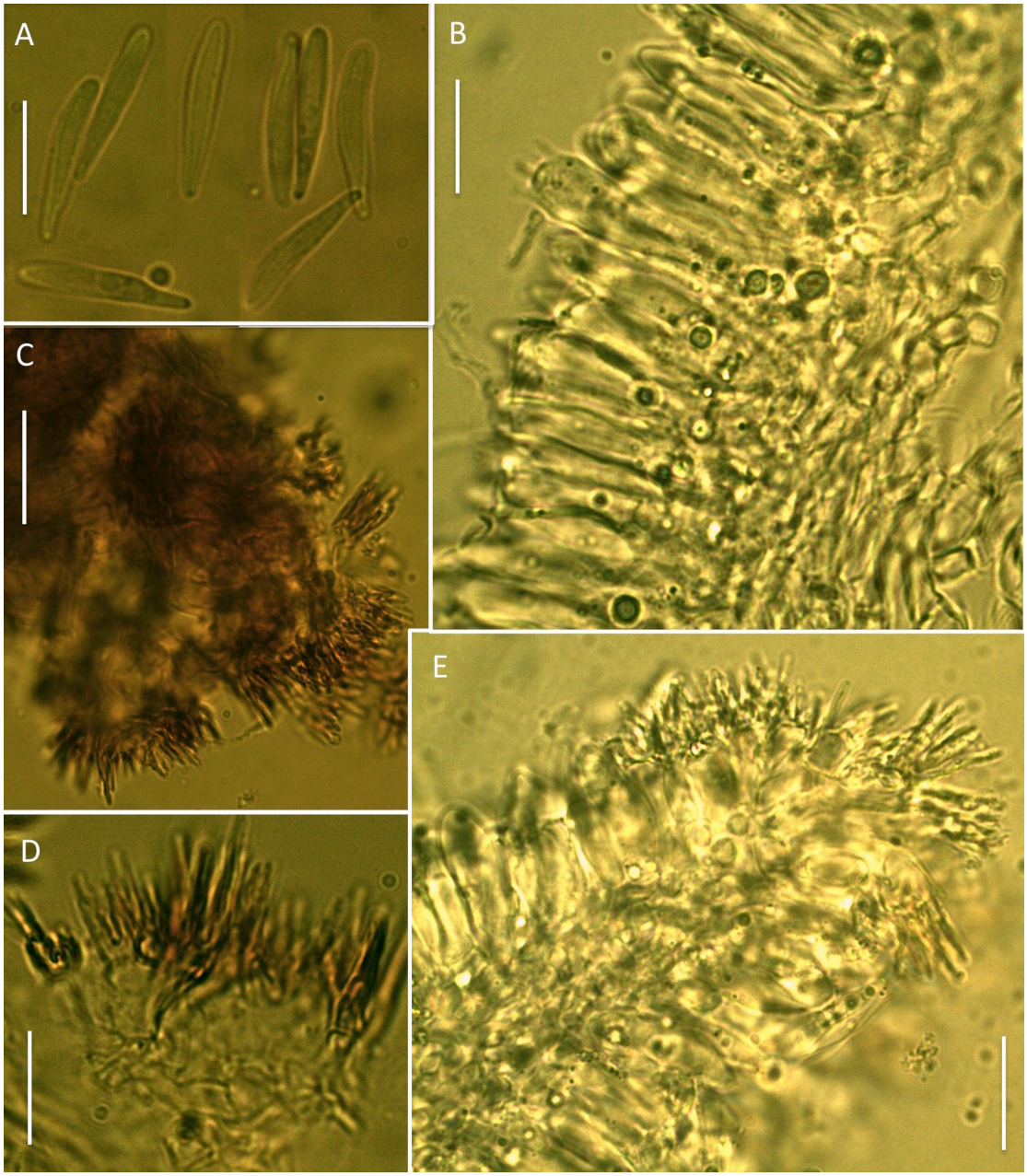


Fig. 29A ***Marasmius*** **cf.** ***lilacinoalbus***

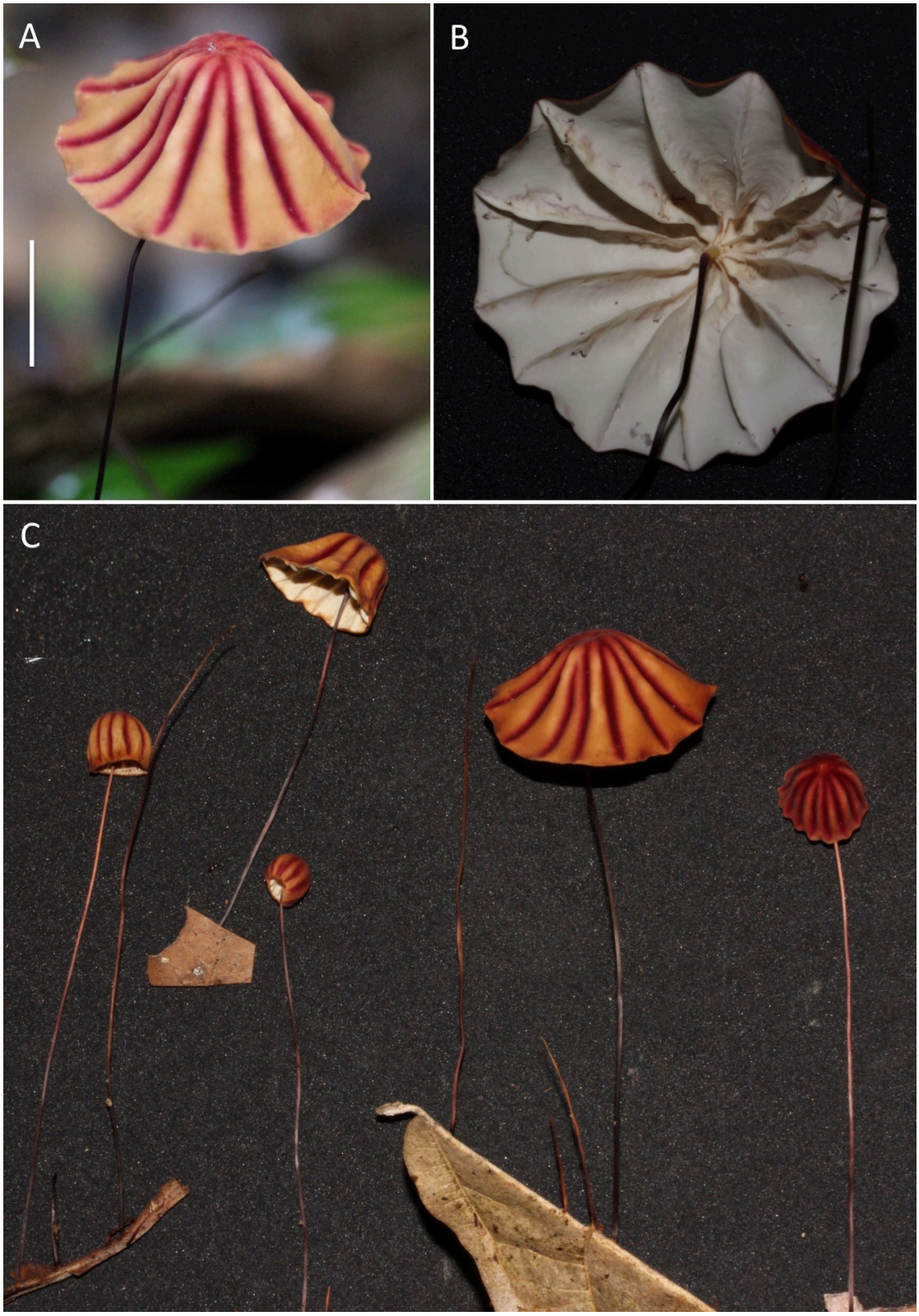


Fig. 29B ***Marasmius*** **cf.** ***lilacinoalbus***

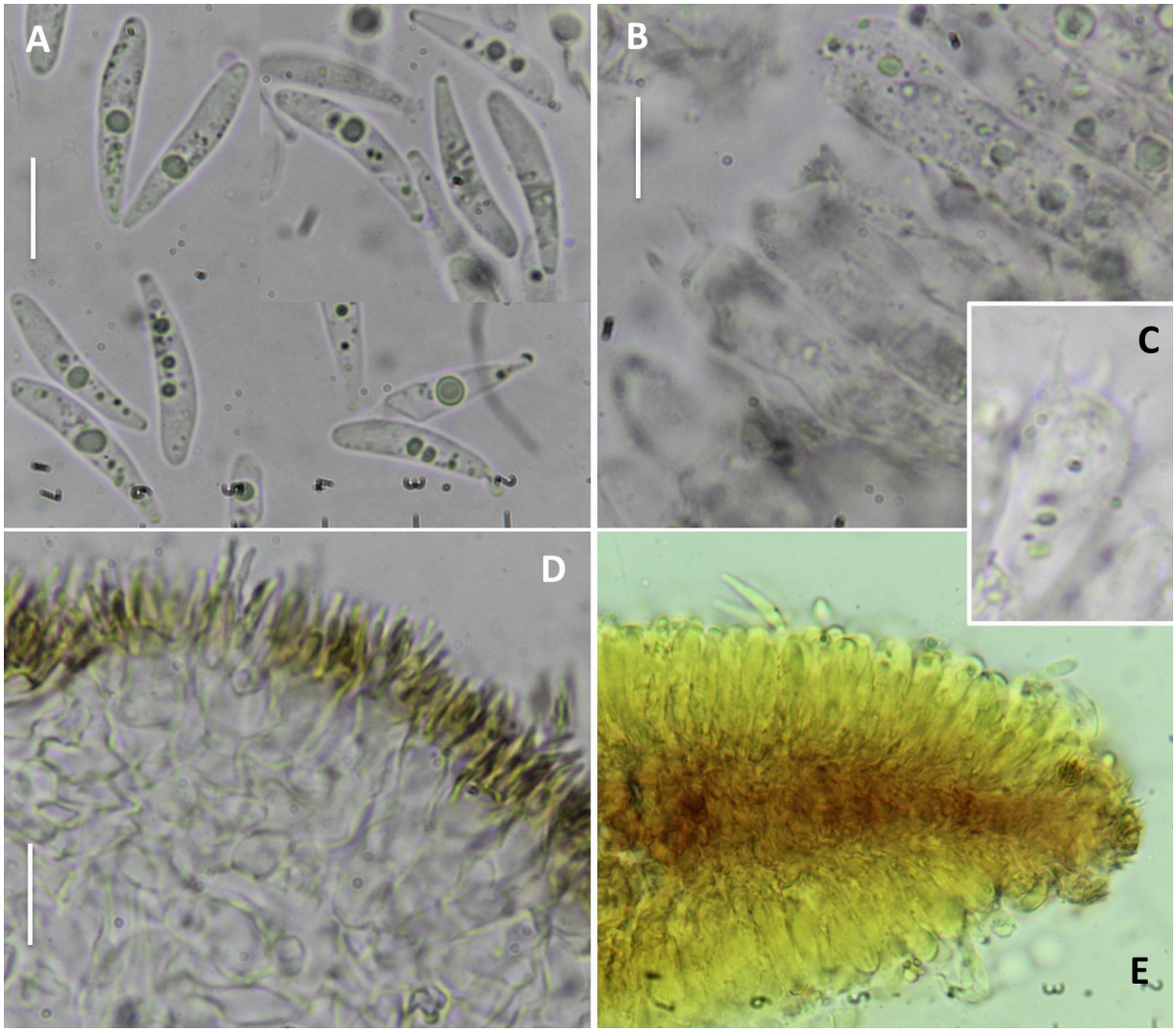


Fig. 30A ***Marasmius leoninus***

Fig. 30B ***Marasmius leoninus***

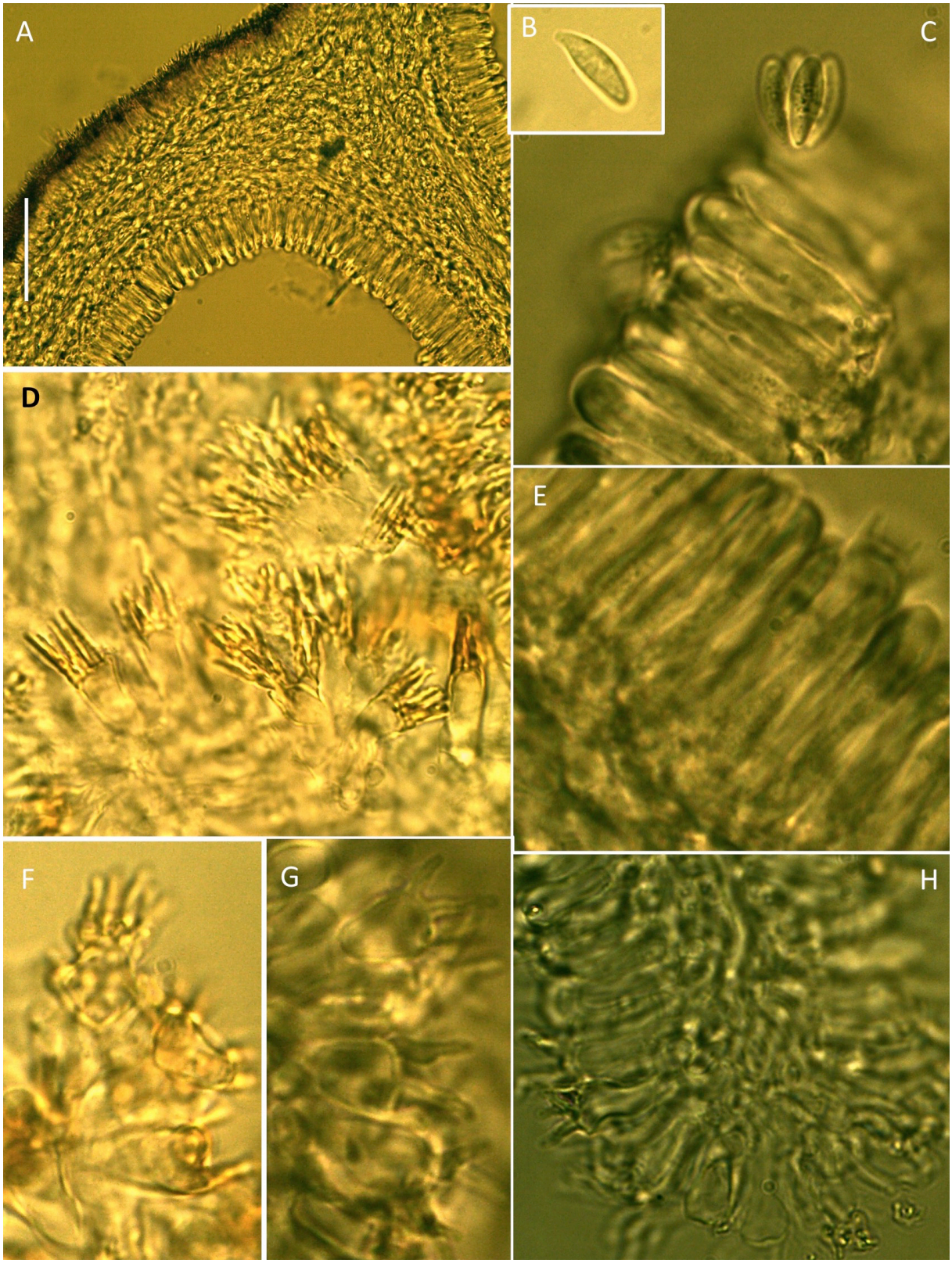


Fig. 31A ***Marasmius*** **"orange21"**

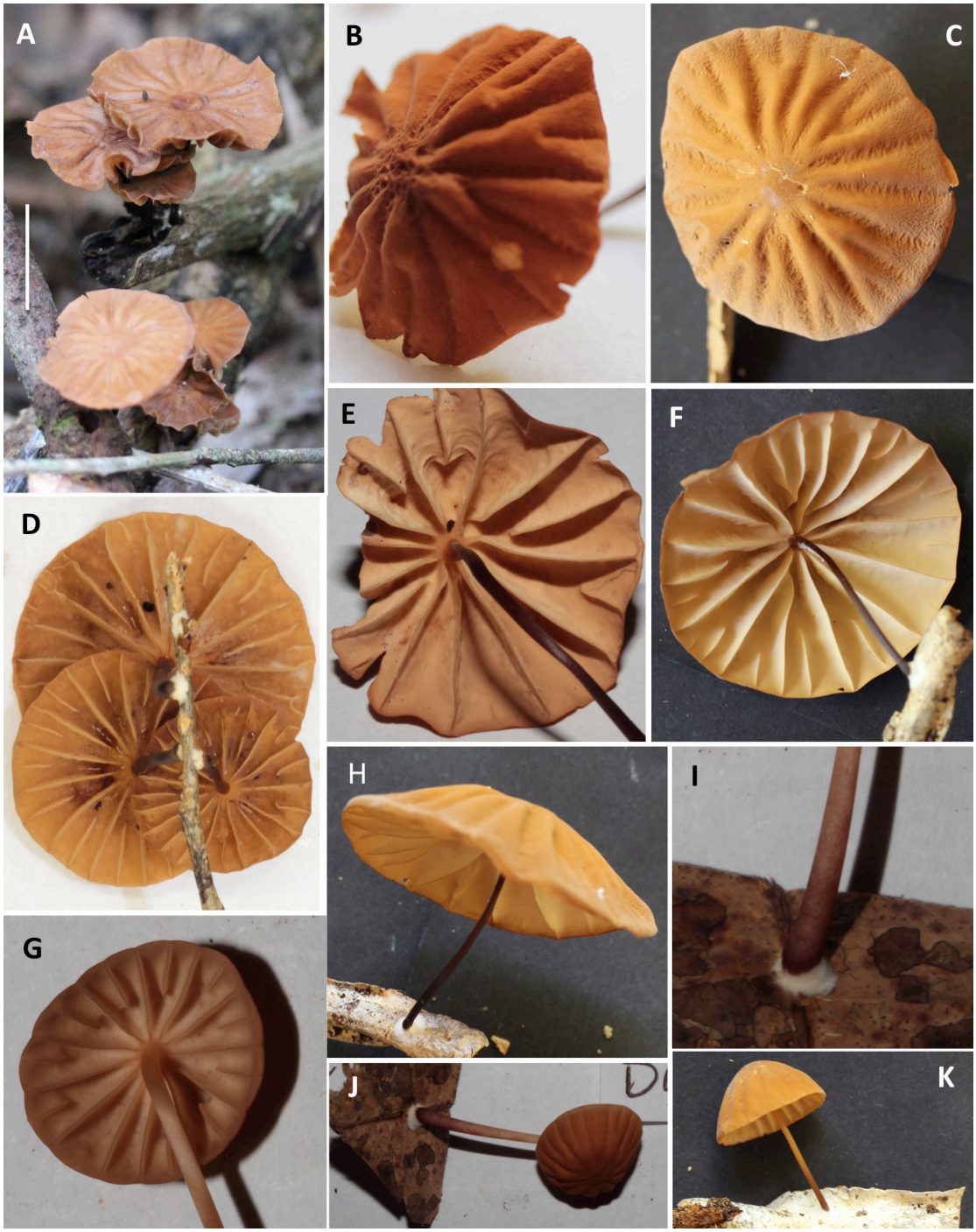


Fig. 31B ***Marasmius*** **"orange21"**

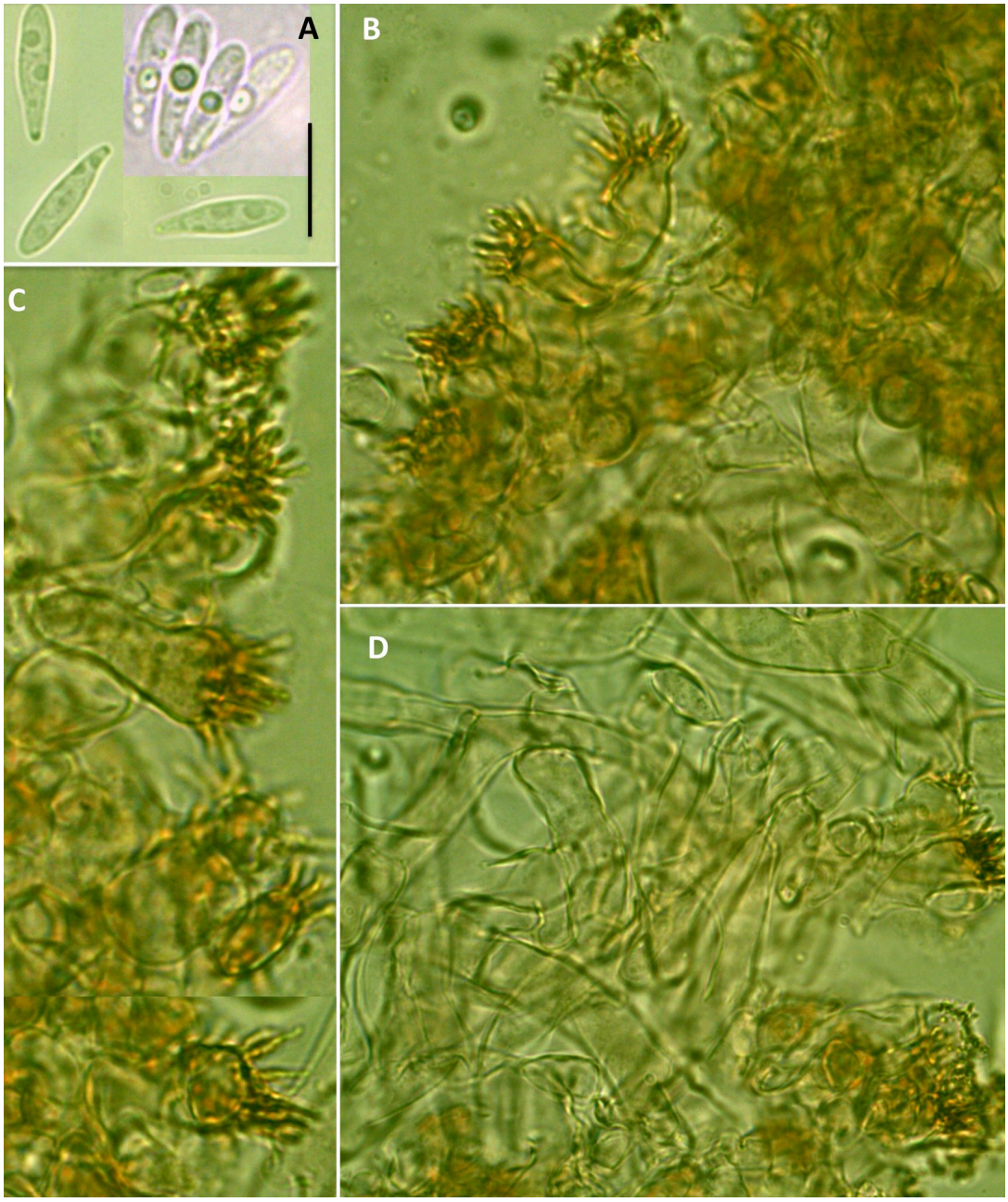


Fig. 32A ***Marasmius jalapensis***

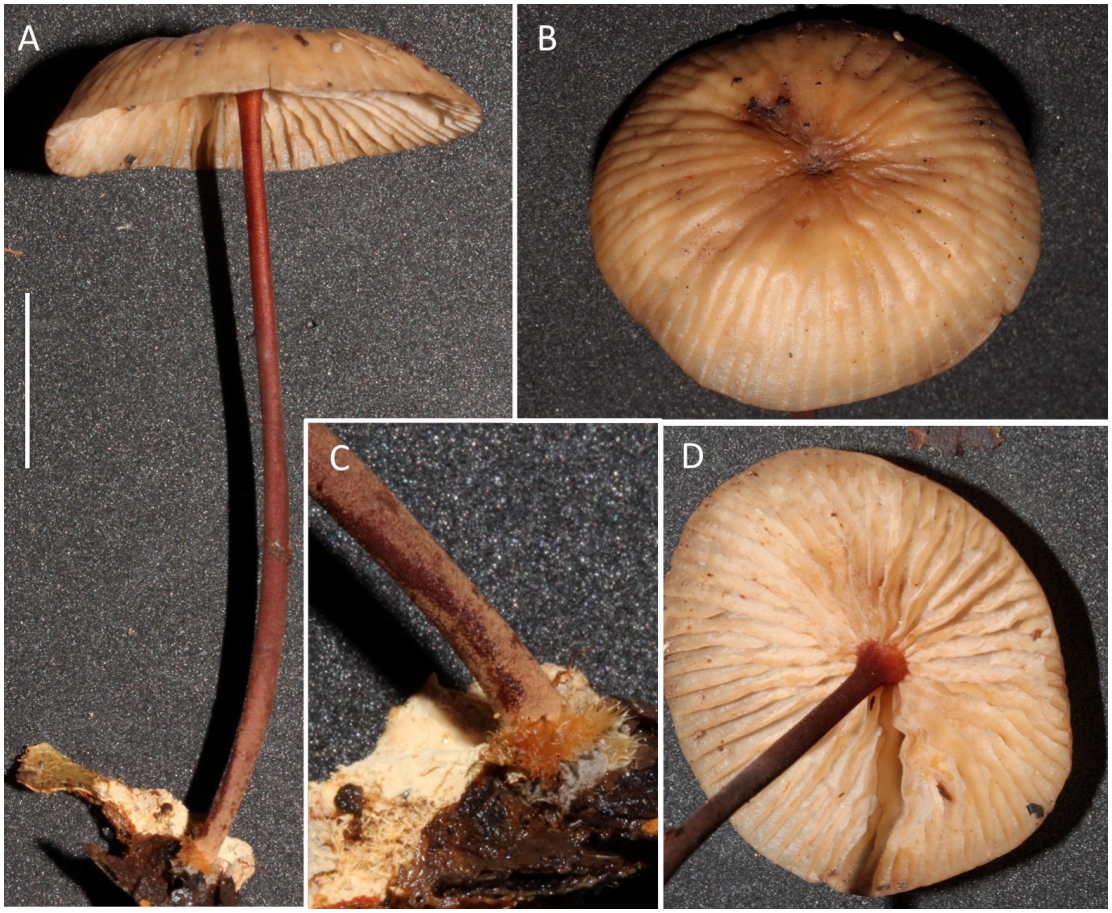


Fig. 32B ***Marasmius jalapensis***

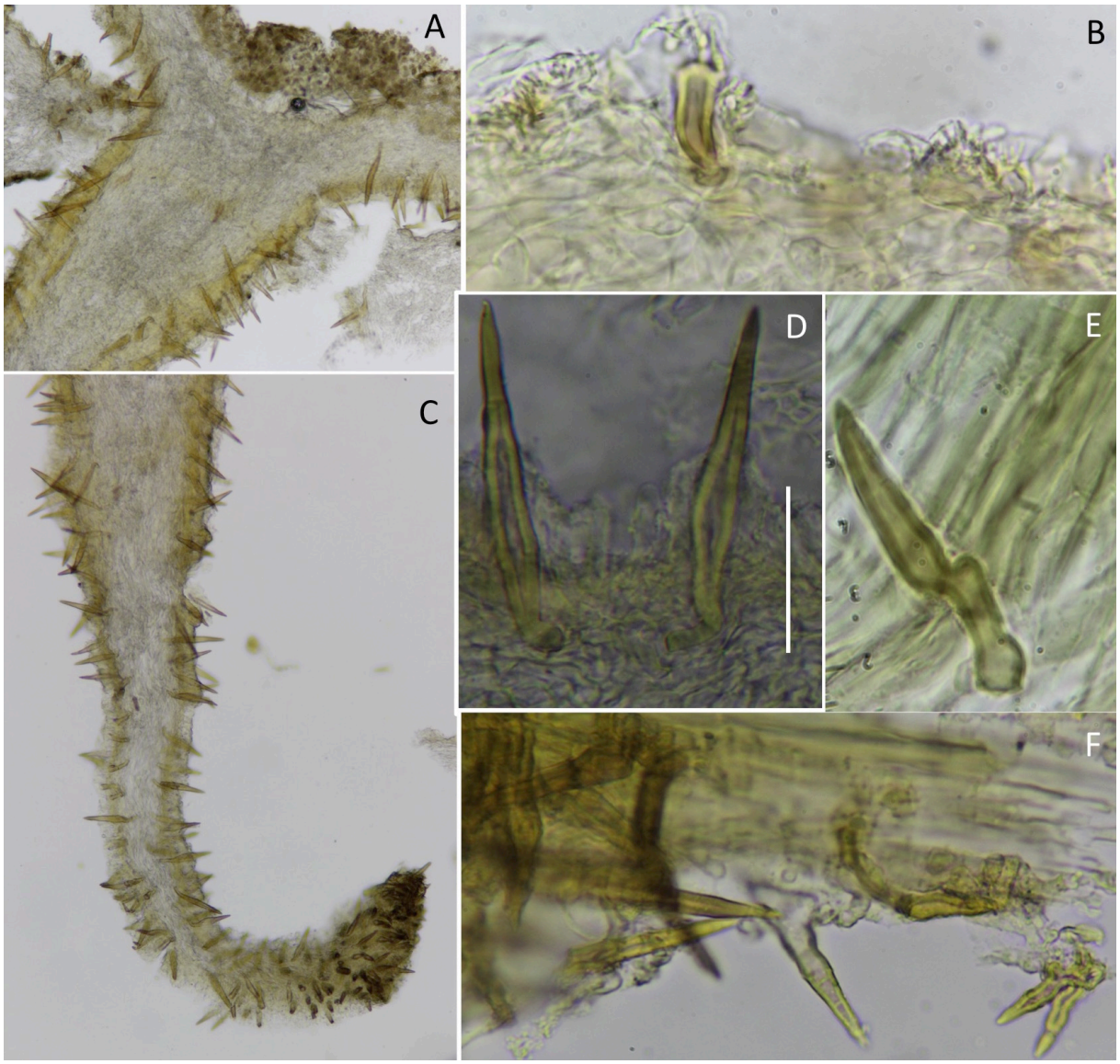


Fig. 33 ***Marasmius*** **"orange3"**

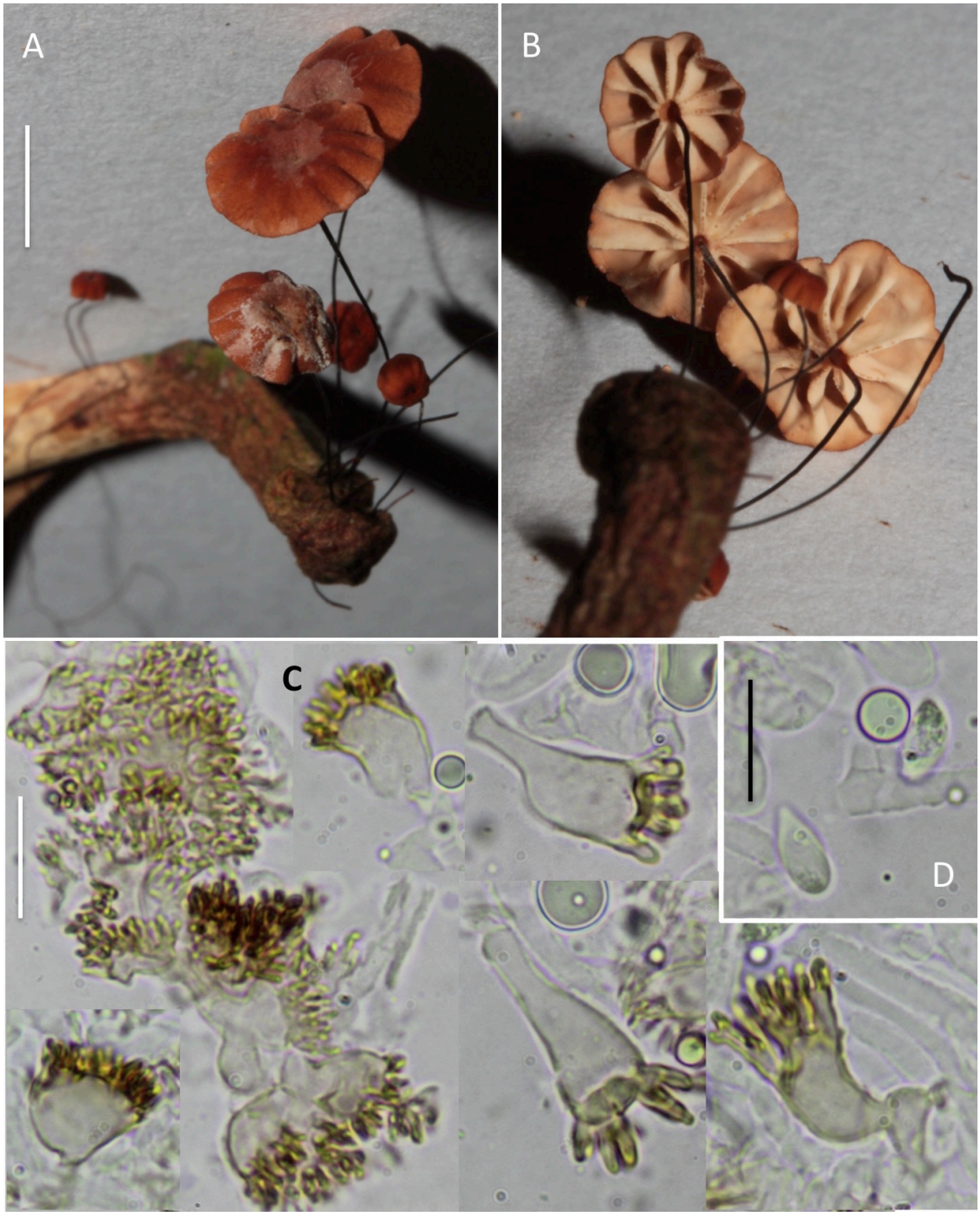


Fig. 34 ***Marasmius*** **"orange16"**

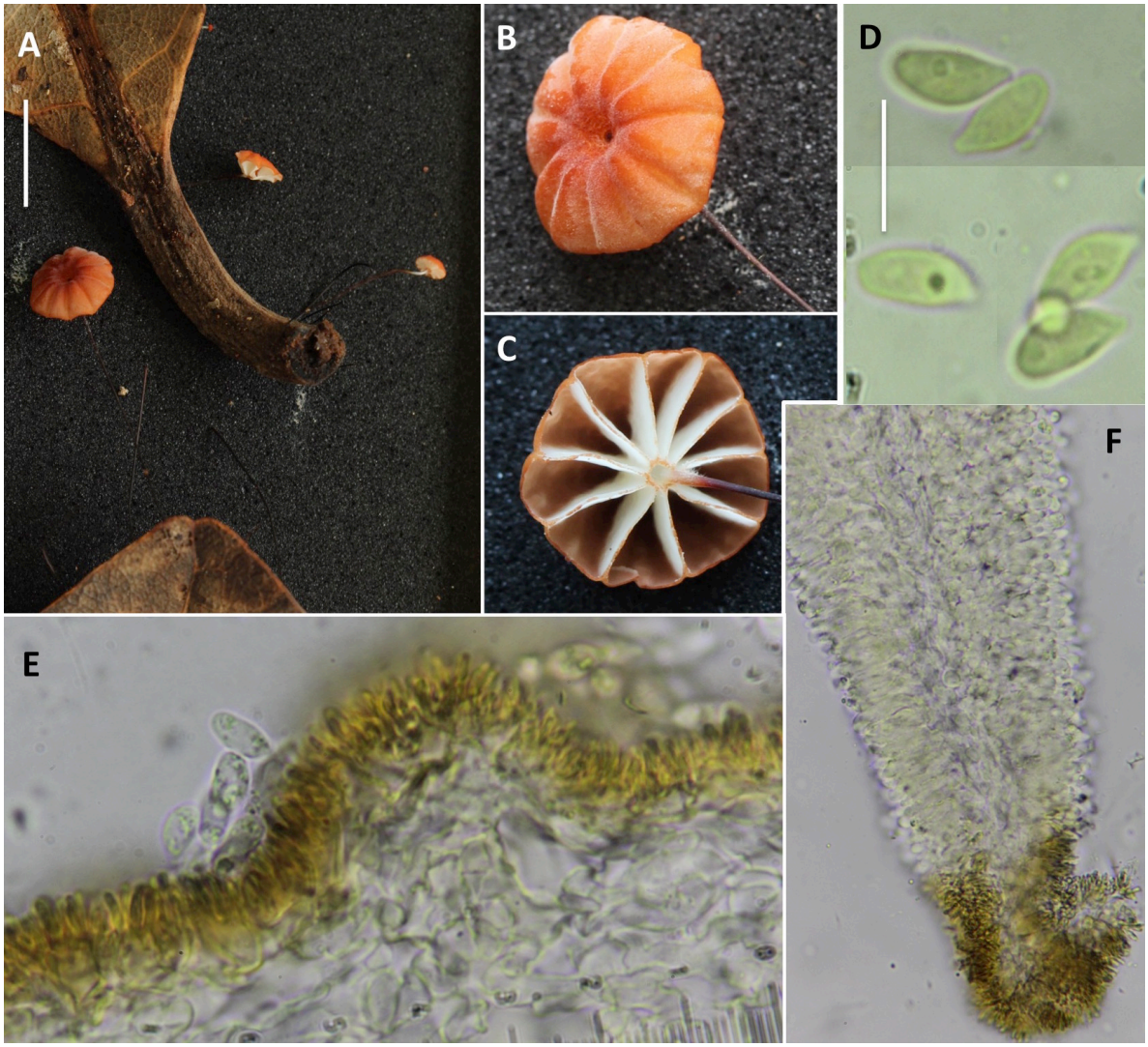


Fig. 35A ***Marasmius*** **"red3"**

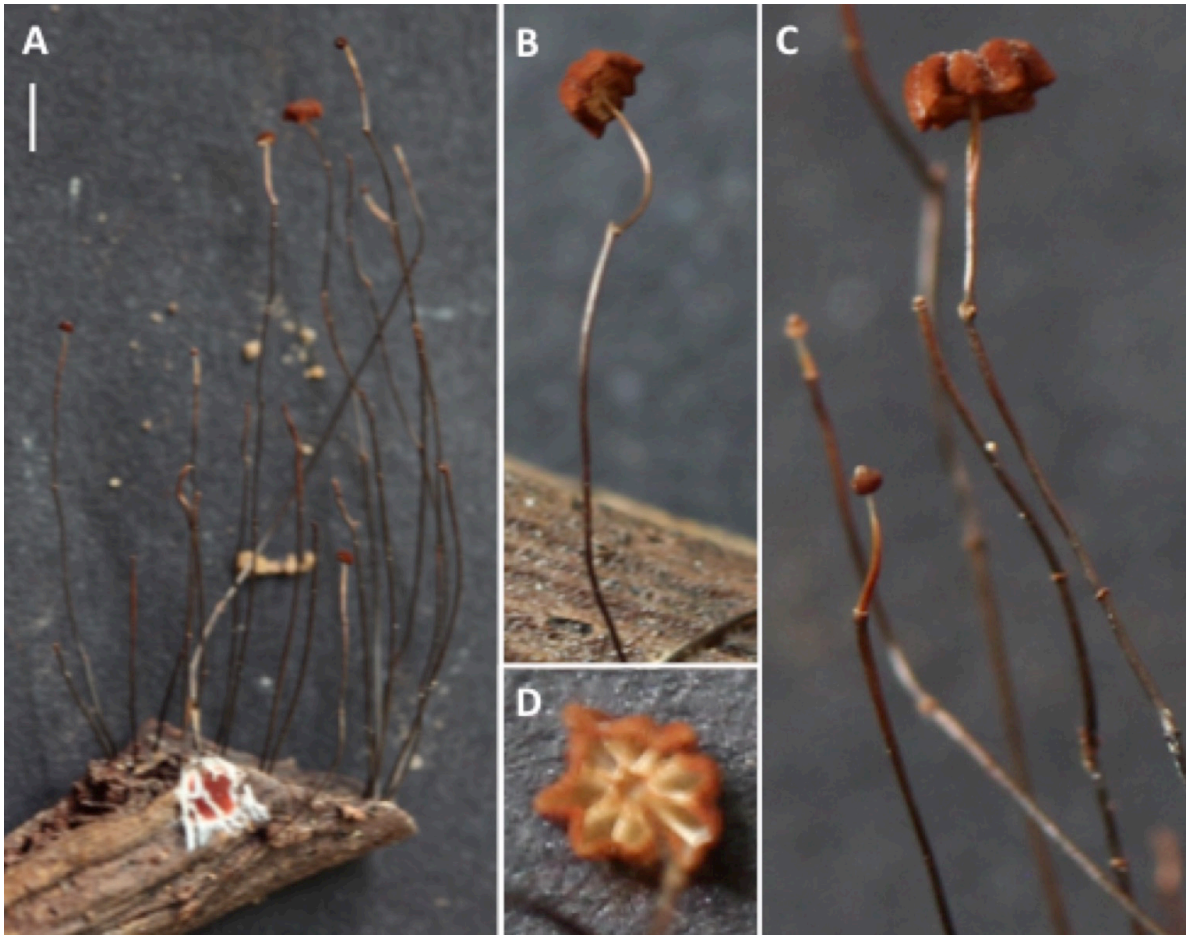


Fig. 35B ***Marasmius*** **"red3"**

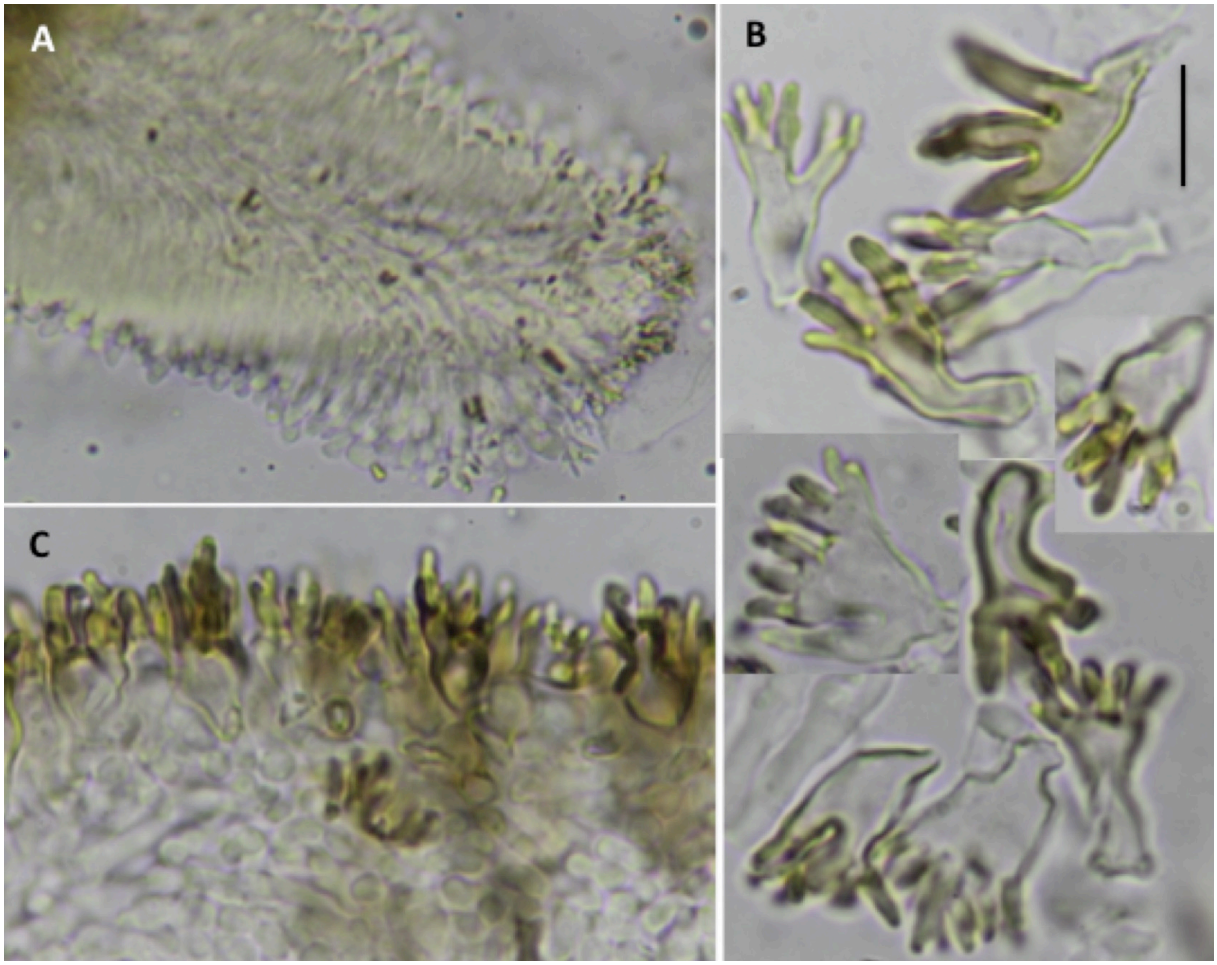


Fig. 36 ***Marasmius rotalis***

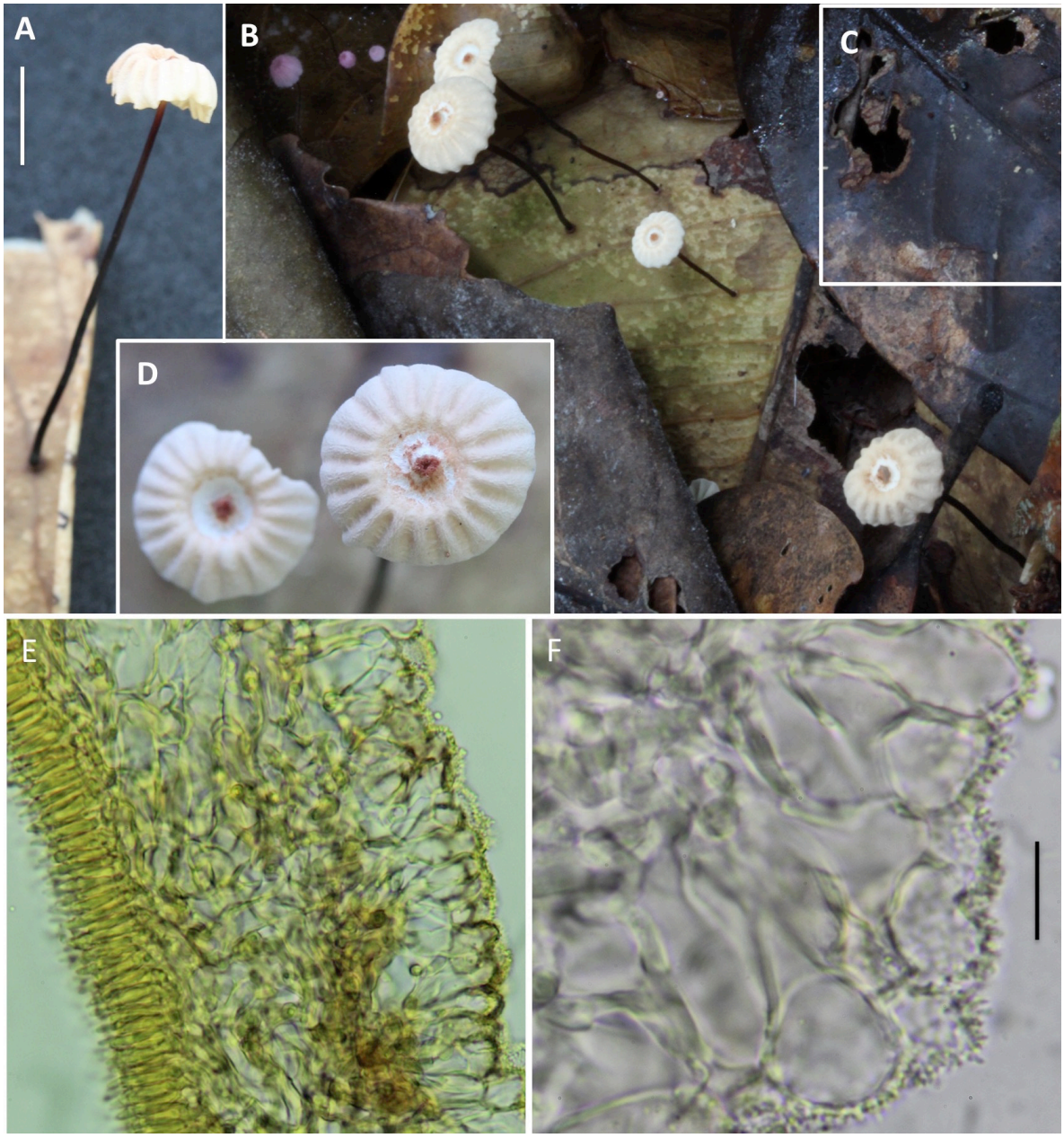


Fig. 37 ***Marasmius castellanoi***

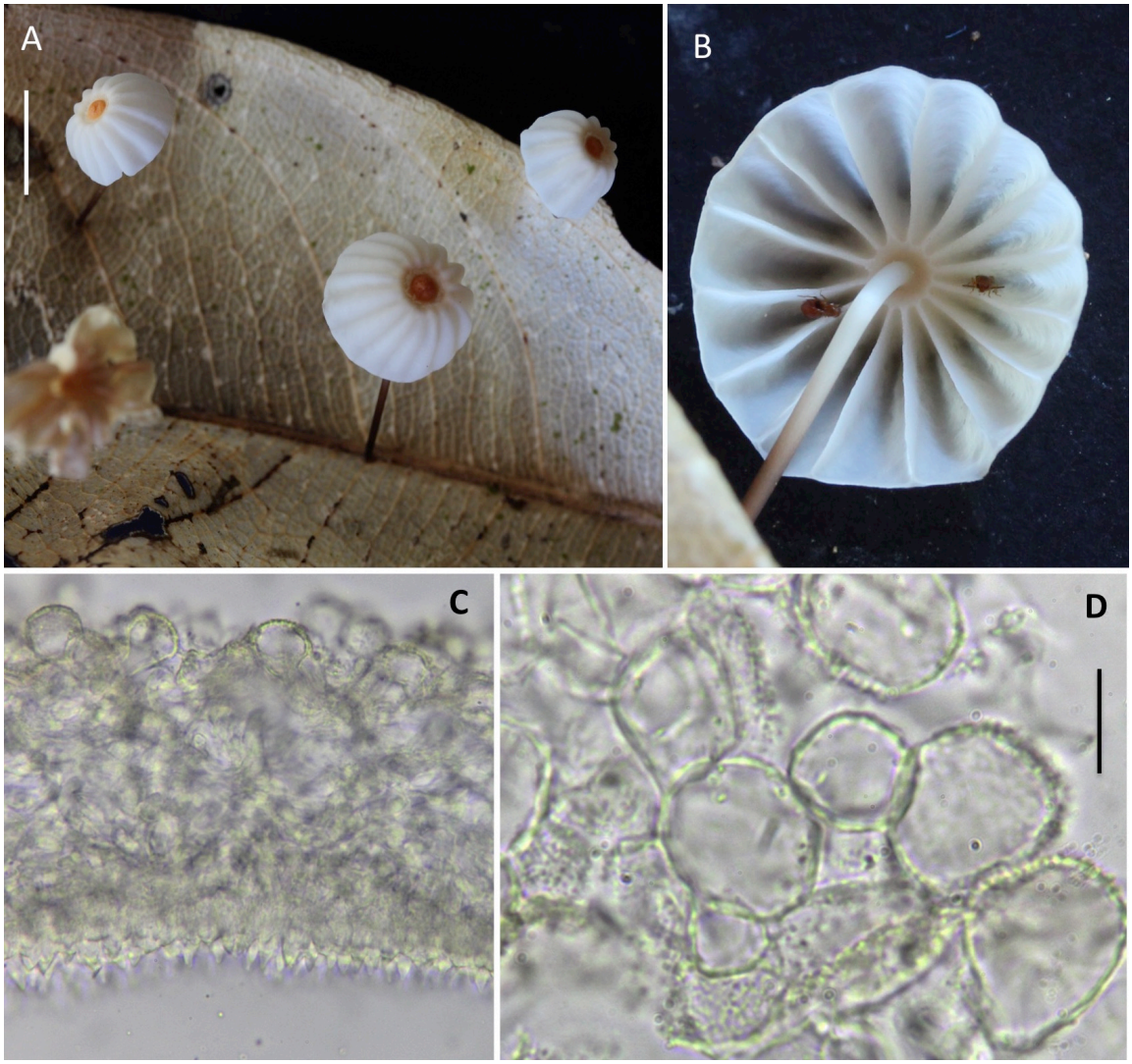
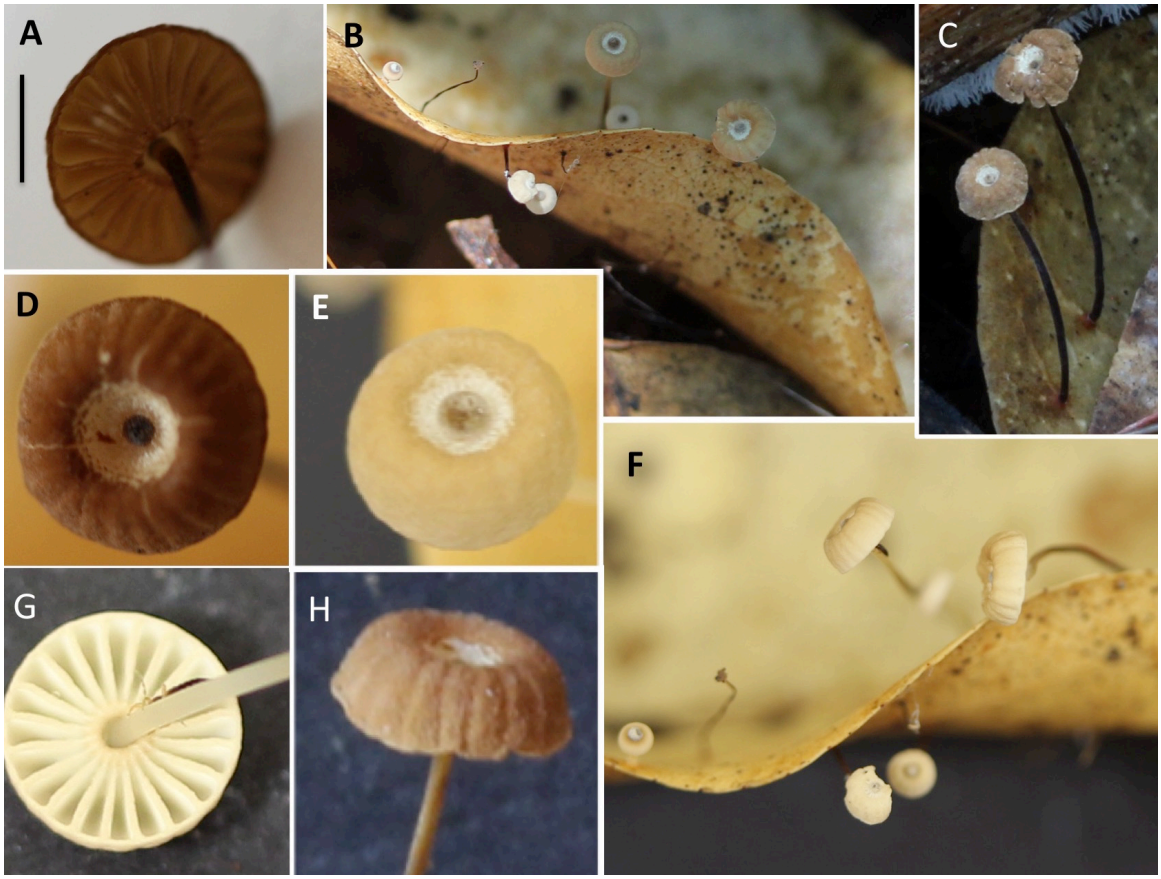


Fig. 38 ***Marasmius scleronematis***



Capítulo 6

Komura, D.L; Moncalvo, J. M.; Margaritescu, S.; Zartman, C. E. *Gymnopus, Rhodocollybia and Marasmiellus* from Amazonian terra firme forest of Brazil.

Manuscrito em preparação para *Acta Amazonica*

Gymnopus, Rhodocollybia and Marasmiellus from Amazonian terra firme forest of Brazil

Komura, D. L.[1*], Moncalvo, J. M. [2]; Margaritescu, S. [2], Zartman, C. E.[1]

[1]National Institute for Amazonian Research, Department of Botany, C.P. 478, 69011-970 Manaus, Amazonas, Brazil

[2]Department of Natural History, Royal Ontario Museum & Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

Department of Natural History, Royal Ontario Museum, Toronto, Canada

National Institute for Amazonian Research, Department of Botany, C.P. 478, 69011-970 Manaus, Amazonas, Brazil

* E-mail address: leimybio@gmail.com

Abstract

Twenty-three taxa of Omphalotaceae group are reported representing fifteen species of *Gymnopus*, seven *Marasmiellus* and one *Rhodocollybia* from Brazilian Amazon. Descriptions, illustrations, photographs from taxa are provided. Molecular phylogenetic reconstructions are presented based on ITS regions in maximum likelihood analyses. Phylogenetic data were strongly correlated with morphological data and were useful to aid in delimiting species and distinguishing among closely related species.

Key words: Agaricales, *Marasmiellus volvatus*, Neotropic, taxonomy

Introduction

Gymnopus (Pers.) Roussel, *Marasmiellus* Murrill and *Rhodocollybia* Singer are saprotrofic fungi and currently belong to Omphalotaceae (Matheny et al. 2006), however their phylogenetic relationship still unclear and studies to better understanding are underway (Moncalvo et al 2002, Wilson and Desjardin 2005, Mata et al. 2006).

Rhodocollybia is reported infrequent in Central and South America, when compared with North temperate America and Europe (Halling 1989; Mata et al. 2004), according to Mycobank 49 legitimate names are associated to this genus. Singer (1939) proposed the genus name *Rhodocollybia* for those species in *Collybia*, but keeped in the *Collybia* section Striipedes in subsequent works (1976), basically this group was characterized with more or less pink spore print, spores often pseudoamyloid and cyanophilic; stipe often rather thick, more or less longitudinally striate or sulcate. Mata et al. (2004) described *Rhodocollybia* from montane forest from Venezuela, Bolivia and Costa Rica, in Brazil just is known the record of *R. butyraceae* (as *Collybia butyraceae* at SpeciesLink http://www.splink.org.br).

Gymnopus in morphological sense is characterized by basidiomata marcescent, tough, or membranous, rarely putrescent and fleshy and rarely originating from a sclerotium; spore deposit white to cream colored and spores inamyloid, acyanophilous, pileipellis a cutis or trichoderm, made of hyphae with none to few or numerous projections, or with lobed to diverticulate terminal elements, frequently with incrusting pigments (Halling 1997, Antonín et al. 1997), they correspond the sections *Iocephalae*, *Levipedes* and *Vestipedes* from *Collybia* described in Singer (1962). Nooderloos and Antonín (2008) based in phylogenetic studies carried out by Mata et al. (2004), Matheny et al. (2006) and Wilson and Desjardin (2005) transfer the *Marasmius* sect. *Androsacei* Kühner to *Gymnopus* sect. *Androsacei*. This is one of the changes that are going on this huge genus (331 legitimate name are associated with this genus at Mycobank), with broad distribution, also in the tropics.

In relation to *Marasmiellus*, is almost cosmopolita, more diverse in the tropics (Singer 1962), for the Neotropical area, Singer (1973) described 134 species. Traditionally encompasses fungi characterized macroscopically by collybioid or omphalinoid basidiomes with white, yellowish, pinkish or brownish pilei and insititious stipes usually pale at apex and darkening towards the base. Microscopically, members of *Marasmiellus* form hyaline, smooth, thin-walled and inamyloid spores, cheilocystidia are often present while pleurocystidia, are usually absent, and the pileipellis sometimes with transition to a trichoderm, with or without a well-developed *Rameales*-structure (Singer 1973, 1986). Due

the lack of good character to delimite this genus, is not surprise that currently work have shown its polyphyletic nature (Mata et al. 2004, 2006; Wilson and Desjardin 2005).

Gymnopus and *Marasmiellus*, plus *Marasmius* play an important role to nutrient recycling from necromass, since these species are frequently found (pers. obs.) at poor soil nutrient Amazon terra firm forest (Stark and Jordan 1978, Stark 1982). So the study of this understudied group of fungi is necessary to better understanding its relationship in this forest. On way to do this, is the inclusion of these tropical fungi in the taxonomy, systematic and broad phylogenentic studies. Here we are providing description, ITS data, color image to support future work related to these fungi.

Material and methods

Fieldwork.— Specimens were collected during field expeditions in 2012-2015 at six sites in the Brazilian Amazon: (1) Estação Experimental de Manejo Florestal do INPA (ZF-2) (02°37’ S, 60°09’ W), about 80 km north of Manaus, the state capital of Amazonas; (2) Reserva de Desenvolvimento Sustentável do Tupé (3° 07' S, 60° 18' W), 25 km west of Manaus on the banks of the Rio Negro; (3) Reserva Biologica Ducke of INPA (2° 58' 48" S, 60° 09' 08" W) in the neighborhood of Manaus; (4) Reserva Biológica do Uatumã in Balbina, north from Manaus (S1°47'21" S, 59°15'08" W); (5) Parque Nacional do Viruá located in Caracaraí, Roraima State (1°28'05"N , 61°00'32" W) and (6) Floresta Nacional do Tapajós (2° 51' 37" S, 54° 57' 57" W) at ICMBio Station Base Terra Rica, located at km 67 and at Jamaraquá Communit (2° 49' 45" S, 55° 01' 56" W), Belterra, Pará State.

Morphological descriptions.— Freshly collected specimens were described macroscopically, and digital images were taken. We used the color code based on Color Picker chart color (http://www.colorpicker.com). Collections were dried at 40-50 °C with the use of an electric dehydrator (A. & J. Stöckli) or in silicagel for subsequent microscopical examination and herbarium preservation. The microscopic observation was carried out according to Oliveira and Capelari (2012). Sections of dried material were rehydrated in 70% ethanol and mounted in 5% KOH or in Melzer reagent for the amyloidity test. The dimensions of the spore measurements included the range of length × width, and following statistical analysis: X_m, the arithmetic Mean of length × width ± standard deviation; Q_m, the mean of the range of length/ width of basidiospores ± standard deviation; and n, the number of spores measured. The lamellae spacing was determined by the following factors: L, the number of lamellae that reach from the stipe to the pileus margin; and l, the number of series of lamellulae among the

lamellae. The dried collections were deposited in the INPA and duplicates in the Royal Ontario Museum Fungarium (TRTC).

ITS sequences production and analysis.— DNA isolation, PCR amplification, sequencing and editing of the ITS region followed Dentinger et al. (2010). Sequences showing >90% similarity to the newly produced *Marasmius* sequences were retrieved from BLAST (Altshul et al. 1990) searches in the NCBI database (GenBank), and aligned with our sequences using MUSCLE (Edgar 2004). A preliminary analysis was conducted in MEGA version 6 (Tamura et al. 2013) using Maximum-Likelihood (ML) and default parameters. From this preliminary analysis (data not shown) we selected 99 ITS sequences (Table S1) that were deemed to be relevant for this study, we do not used outgroup. ML settings for the final analysis were determined in jModeltest 2.1.7 (Darriba et al. 2012). Bootstrap support (BS) for branches was estimated from 1,000 replications. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (-10833.2415) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.7717)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 29.1399% sites). The final analysis involved 99 nucleotide sequences. All positions with less than 90% site coverage were eliminated. That is, fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 577 positions in the final dataset.

Results and discussion

The maximum likelihood analysis for all ITS sequences is given in Figure 1. These preliminary results showed some tendency to split species in three major groups, *Gymnopus* group, *Rhodocollybia* group and *Marasmiellus*, but with low support. However is known that *Gymnopus* and *Marasmiellus* are polyphyletic groups (Moncalvo et al. 2002, Wilson and Desjardin 2005, Mata et al. 2004, 2006).

The group labeled as "*Marasmiellus* group" here with moderate support (78% BS), presented *Tetrapyrgos* sp. close related to one *Marasmiellus* clade with 100% BS, another group of *Marasmiellus* are split without support. Wilson and Desjardin (2005) also found similar result, where the *Marasmiellus candidus* (type species from section Candidi) placed

among *Tetrapyrgos* species. The identity of *Tetrapyrgos* is warranted by tetrahedral or with conspicuous lateral bulge basidiospores while *Marasmiellus* member, basidiospores are smooth. However, Moncalvo et al. (2002) results showed *Tetrapyrgos* is closer related to *Campanella*, among the Marasmiaceae, as Matheny et al. (2006), while Bodensteiner et al. (2004) and Wilson and Desjardin (2004) suggested *Tetrapyrgos* within Omphalotaceae. At the moment, this is unresolved issue.

In relation to *Rhodocollybia* group, all the sequences labeled as *Rhodocollybia* were placed in the same clade, but without support. The sequences from *Rhodocollybia* sp.1 are grouped with high support (100%).

About the *Gymnopus*, here represented with majority of the sequences, we could not find any pattern in the group according to morphology, which could be represent the subsection in *Gymnopus*, but it was usefull to identify especimens with similar morphology. Other comments are in the descriptions comments for each taxon.

Taxonomy

1- *Gymnopus* aff. *parvulus*

Figure 2

Pileus 2–4 mm diam, broadly convex, with a depresed disc, sulcate margim striate, smooth to slightly pruinose, dull, opaque cream (F0DBB9) to apricot brownish at the disc (BF8162); **context** thin, cream. **Lamellae** adnate, subdistant, *L*= 13–15, *l*= 1–2 series, cream, turning brownish at center in age edge concolorous with lamellae. **Stipe** 3–5 × 0.5 mm, central, wiry, opaque, felted, insititious, brown to whitish to upward. Superficial mycelia under the leaf present.

Basidiospores not observed. **Basidia** not observed. Basidioles 20 × 5 μm, clavate, thin-walled, hyaline. **Cheilocystidia** main body 15–20 × 5 μm, elongate, lobate, branched, irregular, thin-walled, hyaline. **Pleurocystidia** absent. **Pileipellis** prostate, thin-walled, hyaline cells, irregular. **Pileus trama** 4 μm diam. interwoven. **Lamellar trama** interwoven, hyaline. **Stipitipellis** paralles cells, thick-walled, brownish. **Caulocystidia** elongated, thich-walled, hyaline. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmioid, gregarious on branch and leaf at leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1284 (INPA259617), DLK1287 (INPA259618), DLK1289 (INPA259619).

Comments: According to ITS phylogenetic tree, our three sequences of *Gymnopus* aff. *parvulus* are clustered together with high support (100 % BS) and are close related to *Gymnopus parvulus* (Mata et al. 2006) with moderate support (88 % BS). In relation to morphological characters, the pileus size of *G. parvulus* is larger than 10 mm, while our specimen just reaches 4 mm diam and the pileocystidia are somewhat similar among the species.

2- *Gymnopus* sp. 16

Figure 3

Pileus 7 mm diam, broadly conic, pale brown (915D01); **context** thin, cream. **Lamellae** adnexed, close, *L*= 17, *l*= 3 series, margin crenate, concolorous, cream. **Stipe** 20 × 0.8, cylindrical, opaque, pruinous, brownish turning crema upward, attached to substrate by pilose mycelial pad.

Habit, substrate and known distribution: leaf at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 May 2012, D.L.Komura & F.C. Batista, DLK707 (INPA 259692).

Comments: Due the lack of information about microscopic features we could not infer about the identity of this specimen, but clearly the ITS analysis showed that is close related among *Gymnopus* spp.

3- *Gymnopus* sp. 2

Figure 4

Pileus 4-7 mm diam, semi-circular to flabelliform, slightly depressed, margin uplift, smooth, opaque, slightly pearly, peach color (F2C3AE); **context** thin, pink. **Lamellae** attached to collar, distant, *L*= 9-10, *l*= 1 series, edge rough, concolorous with pileus. **Stipe** 1 × 0.5 mm, lateral, reduced, almost inconspicuous, cylindrical, dull, slightly hygrophanous, brownish, insititious.

Basidiospores absent (basidiomes immature). **Basidia** 25–27 × 7–8 μm, clavate. **Basidioles** 23–25 × 5 μm, clavate, acuminate. **Cheilocystidia** main body 40 × 4 μm, elongate, lobate, irregular, hyaline, thin-walled. **Pleurocystidia** absent. **Pileipellis** formed of prostate hyphae, parallel. **Pileocystidia**, not observed. **Pileus trama** interwoven, thin and thick-walled, some with incrustations. **Lamellar trama** interwoven, thin-walled, hyaline. **Stipitipellis** irregular, crowded with caulocystidia. **Stipe trama**, around 3.5–4 μm diam, parallel, thick-walled, brownish, clamped. **Caulocystidia** similar to Cheilocystidia, elongate, lobate, irregular, bifurcate, scant diverticula. **Clamp connection** present in all tissues.

Habit, substrate and known distribution: pleurotoid, gregarious on branch in the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 Apr. 2013, D.L.Komura & O.F. Menezes, DLK1474 (INPA259661).

4- *Gymnopus* sp. 1

Figure 5

Pileus 25–80 mm diam, broadly convex, plane to uplift, margin irregular, incurved to crenate, smooth, dull, opaque cream (F2D5A7) to whitish (F5E7D0) with brownish (946418) to brownish red (75301D) disc in age; **context** thin, cream. **Lamellae** adnate to bulb-like stipe, subdistant, *L*= 12–17, *l*= 3 series, intervenose, concolorous with pileus. **Stipe** 30–40 × 4–5 mm, central, opaque, pruinose cream covering the stipe, more evident in young specimens at the base of stipe, white mycelial pad present, brown to cream to upward, turning dark brown in age.

Basidiospores 6.0–9.0 × 4.0–5.0 μm [χ_m= 7.5±0.8 × 4.3±0.4 μm; Q_m= 1.8±0.2; n=20], elliptic to lacrimoid, thin-walled, inamyloid, hyalin. **Basidia** 22–25 × 6–8 μm, clavate, thin-walled, hyaline, 2-sterigmate. **Basidioles** clavate, some fusiform, thin-walled, hyaline. Cheilocystidia elongated, lobate, irregular, thin-walled, hyaline. **Pleurocystidia** absent. **Pileipellis** irregular, slightly gelatinous, some cells projected, some incrusted, brownish. **Pileus trama** 6 μm diam, interwoven, thin-walled, hyaline. **Lamellar trama**, interwoven, cells with 3 μm diam, thin-walled. **Stipitipellis** parallel, brownish, thick-walled. **Caulocystidia** matted formed by long, branched, lobate, irregular cells, thick-walled. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: branch on leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus,

Jardim Botânico Ducke, 25 Apr. 2012, D.L.Komura & J.M. Moncalvo, DLK376 (INPA271930); Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S.Marinho, DLK511 (INPA259710).

Comments: This specimen showed macroscopic variable morphology, more brownish cream and with numerous veins and turning whitish brownish and with less veins in age. ITS analysis showed no differences between the two sequences. Despite this species is clusted among *Gymnopus* spp., some characteristics are shared with *Marasmiellus*, and more accurate microscopical analysis is necessary to better delimit this genus.

5- *Gymnopus* sp. 3

Figures 6a and 6b

Pileus 23-35 mm diam, plane convex, slightly depressed sulcate margin striate, pearly, moist cream whitish (F0E9D8) with apricot (D1B08C) disc turning brownish wit age; **contex**t thin, cream. **Lamellae** adnate, distant, *L*= 11–12, *l*= 3–4 series, smooth, concolorus with pileus. **Stipe** 10–25 × 2–3 mm, central, cylindrical, slightly felted, opaque, insititious, light brown cream to upward, turning darker in age. **Basidiospores** 6.6–9.0 × 3.3–4.4 µm [χ_m= 7.5±0.6 × 3.9±0.3 µm; Q_m= 1.9±0.2; n=20], elliptic to lacrimoid with apiculus, inamyloid, thin-walled, hyaline, with drops, mostly with a single large drops. **Basidia** 16–29 × 6.1–6.8 µm, clavate, 4-sterigmate. Basidioles 20–25 × 4.4–4.7 µm, clavate, cylindric, some afilate, thin-walled, hyalin. **Cheilocystidia** main body 28–40 × 4.0–5.8 µm, elongate, lobate, thin-walled, hyaline. **Pleurocystidia** absent. **Pileipellis** irregular, prostrate, parallel cells. **Pileocystidia** not observed. **Pileus trama** similar to lamellar trama. **Lamellar trama** 4–5 µm diam, interwoven, thin-walled, hyaline. **Stipitipellis** irregular, some finger-like projections. **Stipe trama** parallel cells, thick-walled, brownish. **Caulocystidia** finger like, hyaline. **Clamp connections** present in all tissues. **Odor** sweet smell.

Habit, substrate and known distribution: branch and trunk in the leaf litter at primary and secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1311 (INPA 259654); 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1334 (INPA 259658); 21 May. 2013, D.L.Komura & O. F. Menezes, DLK1426 (INPA259660).

Comments: This taxon is related with specimen DLK1852 from Pará in the ITS analysis, but morpholgicaly is a distint species.

6- *Gymnopus* (DLK1852)

Figure 7

Pileus 110 mm diam, convex, slighty sulcate, margin striate, smooth, dull, brown (7A3506) to pale brownish (D48550); **context** cream, thick. **Lamellae** adnate to adnexed, close, *L*= around 20, *l*= 4 series, thin, smooth, pale brown. **Stipe** 110 × 10 mm, central, cylindrical, hollow, slightly pruinouse and striate, opaque, dull, institious, brownish with white covering.

Basidiospores 8 × 5 µm, elliptic to lacrimoid, thin-walled, inamyloid, hyaline, with drops, apiculate. **Basidia** not observed. **Basidioles** 25–32 × 4–5 µm, clavate, fusiform, thin-walled, hyaline, with many drops. Cheilocystidia not observed. **Pleurocystidia** absent. **Pileipellis** irregular, hair-like cells, hyaline, 2 µm diam and also thick-walled incrusted in strips,

brownish. **Pileus trama** interwoven, thin and thick-walled cells, incrusted in strips, brownish, 5–7 μm diam. **Lamellar trama** interwoven. **Stipitipellis** parallel, thick-walled, 5 μm diam. **Caulocystidia** 20–24 × 4–6 μm, setiform, fusoid, some apex obtuse, hyaline. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: solitary on soil at terra firme forest.

Material examined: Brazil, Pará State, Belterra, Floresta Nacional do Tapajós, Comunidade Jamaraquá, 24 Mar. 2014, D.L.Komura, T.S.Cabral, I.R. Fonseca, DLK1852 (INPA271968).

Comments: The ITS sequences placed this taxon in the same clade of *Gymnopus* sp.3, albeit the both taxa are morphologicaly distint. This species produces large and carnose basidiomes (pileus 110 mm diam *versus* 23-25 mm diam to *Gymnopus* sp.3).

7- *Gymnopus* sp. 13

Figures 8a and 8b

Pileus 5-10 mm diam, plane convex, center slightly depressed, discolorous, pruinose, sulcate, margin discolor, felted, opaque, dull, dark brown (5C2B00) center lighter (BF9571); **context** thin, brownish. **Lamellae** adnate, distant, *L*= 12, *l*= 2–3 series, edge slightly membranose to gelatinous, lamellae brown color edge translucid brown. **Stipe** 1 × 7 mm, central, cylindrical, pubescent, opaque, dull, insititious, black to brown. **Basidiospores** 7.0–10.0 × 4.6–5.0 μm [χ_m= 8.7 ± 1.2 × 4.6 ± 0.5 μm; Q_m= 1.9 ±0.4 μm; n=12], lacrimoid to ellipsoid, smooth, thin-walled, hyaline. **Basidia** 29–31 × 7μm, clavate, 4-sterigmata. **Basidioles** 25–26 × 6–7 μm, clavate. **Cheilocystidia** main body 42–52 × 10–16 μm, clavate lobate, bifurcate, thin-walled, hyaline. **Pleurocystidia** absent. **Pileipellis** significantly pigmented, strongly encrusted in bands with small calluses in profile. **Pileocystidia** main body 36–80 × 18–21 μm, inflate, clavate, pyriform, bulboid, thin-walled, hyaline. **Pileus trama** interwoven, thin-walled. **Lamellar trama** similar cells of pileus trama. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmiod on branch in the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1386 (INPA259683).

8- *Gymnopus* sp. 30

Figure 9

Pileus 10 mm diam, convex, margin striate, pruinose, opaque, dull brown (AB4403), margin cream (FCE1BD); **context** cream, slighty thick. **Lamellae** adnate, close, L= 16, l= 3–5 series,

thin, smooth, cream. **Stipe** 20 × 3mm, central, cylindrical, hollow, pubescent, opaque, dull, insititious, brownish to amber.

Habit, substrate and known distribution: trunk primary

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 23 22 May 2013. D.L.Komura & F.C. Batista, DLK621 (INPAXXX).

Comments: Due the lack of information about microscopic features we could not infer about the identity of this specimen, but clearly the ITS analysis showed that is close related among *Gymnopus* spp.

9- *Gymnopus* sp. 12

Figures 10a and b

Pileus 4-16 mm diam, convex to plane convex, umbonate, papilate margin striate in age, smooth to felted and pruinose, opaque, dull brown (4F2A09) to dark brown (5C2B00), center lighter (BF9571); **context** thin, brownish. **Lamellae** adnate, distant, *L*= 14–15, *l*= 3–7 series, edge lighter than lamellae. **Stipe** 6-15 × 2 mm, central, cylindrical, felted, opaque, dull, insititious, white turning brown in age. **Basidiospores** 8.0–10.0 × 5.0–7.0 μm [χ_m= 8.6± 0.7 × 5.9 ± 0.9 μm; Q_m= 1.5 ±0.2 μm; n=13], lacrimoid to ellipsoid, smooth, thin-walled, hyaline. **Basidia** 32–42 × 7–8 μm, clavate, 4-sterigmate. **Basidioles** 27–31 × 6.5–8 μm, clavate. **Cheilocystidia**, main body 52–110 × 6–18 μm, irregularly clavate, lobate. **Pleurocystidia** ausent. **Pileipellis** irregular brown, with encrusted cells. **Pileocystidia**, main body 5–16 μm broad, very elongated, terminal apice afilate, some bifurcated, encrustated. **Pileus trama** interwoven, thin-walled. **Lamellar trama** similar to pileus trama. **Stipitipellis** brown, thin walled cells. **Caulocystidia** afilated, hyaline, thin-walled. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: collybioid, solitary, branch at leaf litter on primary and secondary terra firm forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 22 May 2012, D.L.Komura & F.C. Batista, DLK611 (INPA259671); 23 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1198 (INPA259675); 24 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1303 (INPA259678); DLK1313 (INPA 259679); 25 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1345 (INPA259681); Reserva de Desenvolvimento Sustentável do Tupé, 06 September 2012, D.L.Komura DLK1069 (INPAXXXX).

Comments: All ITS sequences from this taxon are clustered with high support (100% BS).

10- *Gymnopus* sp. 6

Figure 11

Pileus 5-10 mm diam, broadly convex, uplift, moisture, smooth, margin striate, cream brownish (B89272); **context** thin, cream. **Lamellae** adnexed, subdistant, *L*=11–13, *l*= 4–6 series, cream, smooth, concolorous. **Stipe** 4–10 × 0.5 mm, brownish turnig whitish upward.

Habit, substrate and known distribution: marasmioid, at leaf and branch on leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 11 May 2012, D.L.Komura & T.S. Marinho, DLK566 (INPA259632); 25 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1387 (INPA259644), DLK1388 (INPA259645).

Comments: Due the lack of information about microscopic features we could not infer about the identity of this specimen, but clearly the ITS analysis showed that is close related among *Gymnopus* spp.

11- *Gymnopus* sp. 4

Figures 12a and b

Pileus 4-5 mm diam, plane convex, margin slightly striate, pearly, moist, whitish to cream orange (F09565) disc; **context** thin, white. **Lamellae** adnate, distant, *L*= 8-10, *l*= 3 series, smooth, concolorous with pileus, varing with age, from white to cream. **Stipe** 5 × 0.3 mm, central, translucid, wet, some with minutely pubescence, insititious, light brown cream to upward.

Basidiospores 6.3–9.5 × 3.0–3.7 µm [χ_m= 7.6 ± 1.4 × 3.0 ± 0.6 µm; Q_m= 2.3 ±0.2 µm; n=4], lacrimoid to ellipsoid, thin-walled, inamyloid, hyalin. **Basidia** 19.7-23.5 × 5.7-6.8 µm, clavate, 4-sterigmate, clamped. **Basidioles** 21.1–24.5 × 4.8–6.0 µm, clavate, fusiform, thin-walled, clamped. **Cheilocystidia** main body 15.5–24.5 × 10.4–13 µm, clavate, pyriform with irregular lobes and diverticula at apex, thin-walled, hyaline; diverticula 4–7 × 2–4 µm, irregular, bifurcate. **Pleurocystidia** absent. **Pileocystidia** similar to cheilocystidia. **Pileus trama** interwoven, thin-walled. **Lamellar trama** around 4 µm diam, interwoven, thin-walled. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmiod, gregarious on trunk and branch at leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1385 (INPAXXXX); 21 Jun. 2013, Pereira, M.R.; Ferreira, D.S.; Bento, L.S & D.L. Komura DLK1646 (INPA259665), DLK1697 (INPA259630).

Comments: The ITS analysis placed the sequences of this taxon in the same clade with high support (93%), albeit one of the sequences is divergent, the morphological characters confirm that the three specimens belong to the same species.

12- *Gymnopus* sp. 14

Figures 13a and 13b

Pileus 15–20 mm diam, plane, convex when young, slightly umbonate, sulcate, dry, opaque, dull rust brown (4F2A09) turnig pale brown (A17E5F) in age; **context** thin, brownish. **Lamellae** adnate, distant, *L*= 11–13, *l*= 3–7 series, lamellae concolorous wit pileus. **Stipe** 1–2 × 15–30 mm, central, cylindrical, hollow, pubescent, opaque, dull, insititious, black to cream upward, some sample accompanied by steril rhizomorphs. **Basidiospores** 7.0–11.0 × 4.0–5.0 µm [χ_m= 9.5 ± 1.1 × 4.6 ± 0.5 µm; Q_m= 2.1 ±0.3 µm; n=10], lacrimoid to ellipsoid, smooth, thin-walled, hyaline. **Basidia** 12 × 25 µm, clavate, 4-sterigmata. **Basidioles** 19–36 × 5–8, clavate, lanceolate. **Cheilocystidia** main body 25–30 × 8–17 µm, clavate, diverticulate, diveticula 5–15 × 1–4 µm, bifurcate, obtuse, branched, irregular. **Pleurocystidia** absent. **Pileipellis** cutis with thick-walled, incrustated, brownish cells, some with 20 × 40 µm, inflate, clavate, pyriform, bulboid, thin-walled, hyaline. **Stipitipellis** parallel, thick-walled, brownish. **Caulocystidia** 63–115 × 4–5 µm, hairs with bulboid base, brownish, thick-walled. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmioid, trunk and branches on the leaf litter at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T. S. Marinho, DLK489 (INPA 259668), DLK490 (INPA259669); 25 May 2012, D.L.Komura & F.C. Batista, DLK761 (INPA259672); 23 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1200 (INPA259676), DLK1209 (INPA259677).

Comments: The ITS sequences of this taxon are clustered with high support (100% BS), the morphological features also confirm this pattern. The microscopic characters is somewhat similar in both species, but macroscopic characters are very different, *Gymnopus* sp.14 has a larger, brown pileus (15–20 mm diam) while *Gymnopus* sp.17 is smaller (4-6 mm diam) and the pileus is pale brownish to pinkish.

13- *Gymnopus* sp. 17

Figures 14a and b

Pileus 4–6 mm diam, convex applanate, umbilicate, not papilate, slightly sulcate margin incurved, some plicate, dry, rugulose, opaque pale rose (F5DAD5) to pale browish (D9A95B); **context** thin, brown. **Lamellae** adnate, distant, *L*= 9-11, *l*= 1–3 series, concolorous with pileus, very narrow. **Stipe** 6–15 × 0.5 mm, central, wiry, pubescent, opaque, dull, insititious, black to cream upward.

Basidiospores 9.0–10.0 × 4.5–5.0 µm [χ_m= 9.3 ± 0.5 × 4.9 ± 0.3 µm; Q_m= 1.9 ±0.1 µm; n=4], lacrimoid to ellipsoid, smooth, thin-walled, hyaline. **Basidia** not observed. **Basidioles** 22–25 × 4–5 µm, lanceolate. **Cheilocystidia**, main body 22–25 × 4–8 µm, clavate, diverticulate, some elongated, lobate, bifurcate, diverticula 5–15 × 1–4 µm, bifurcate, obtuse, irregular. **Pleurocystidia** ausent. **Pileipellis cutis** two thickness cell, 5–15 µm, clamped, encrusted. **Stipitipellis** parallel, slightly thick-walled. **Caulocystidia** clavate, some apex thinner. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmioid, close, on the leaf at secondary terra forme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 May 2012, D.L.Komura & F.C. Batista, DLK695 (INPA259690); 28 Jun 2012, D.L.Komura, DLK842 (INPA271940); 25 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1365 (INPA259697); 22 May 2013, D.L.Komura & O.F. Menezes, DLK1491 (INPA271955); 21 Jun. 2013, D.L.Komura, M.R. Pereira, D.S. Ferreira, L.S. Bento, DLK1694 (INPA259699).

Comments: The ITS sequences are very similar, placed all the same clade with high support (100% BS). This species are related with *Gymnopus* sp.14 with high support (100% BS), but macroscopics characters are rather divergent.

14- *Gymnopus* sp. 9

Figure 15

Pileus 5–12 mm diam, convex to plane, irregular, discolor, irregular, incurved, uplift, smooth to finely fibrillose, dry, opaque orange brown (C7630C) to burnt yellow (C4891B); **context** thin, cream. **Lamellae** adnate, close, *L*= 28–30, *l*= 3–5 series, cream. **Stipe** 5–15 × 1mm, central, cylindrical, fibrillose, dry, pubescent, insititious, brownish to cream upward. **Basidiospores** 6.0–8.5 × 3.0–4.5 µm [χ_m= 7.3 ± 0.7 × 3.9 ± 0.3 µm; Q_m= 1.9 ±0.2 µm; n=20], oblong to ellipsoid, thin-walled, hyaline, inamyloid. **Basidia** 20 × 6 µm, clavate, 4-sterigmate, hyaline. **Basidioles** 20 × 5 µm, clavate, fusoid, hyaline. **Cheilocystidia** absent.

Pleurocystidia absent. **Pileipellis** cutis, incrusted cells, some pedunculate. **Pileus trama** interwoven, thin and thick-walled, incrustated in bands. **Lamellar trama** interwoven, thin-walled, hyaline. **Stipitipellis** 4 μm diam, parallel, thick-walled, brownish. **Caulocystidia** 5 μm diam, thick-walled, elongate, apex obtuse, some acuminate, setiform, clavate. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: marasmioid, gregarious on leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1322 (INPA259656), DLK1323 (INPA259657), 26 Apr. 2013, D.L.Komura & P.A.Pereira, DLK1400 (INPA271952), 11 Sep. 2013, D.L.Komura & J.R. Maciel, DLK1757 (INPA271960).

Comments: This specimen is similar to *Gymnopus* sp. 20, but without telepods, both could be the same species. Molecular analysis will be carry out to confirm for this species the presence of telepods is a structure that is presents in some specimens and absent in others.

15- *Gymnopus* sp. 20

Figures 16a and b

Pileus 5–10 mm diam, plane convex, plane, irregular, discolor, striate, irregular, incurved, uplift, smooth to finely fibrillose, dry, opaque orange brown (C7630C) to burnt yellow (C4891B); **context** thin, translucid. **Lamellae** adnate, close, *L*= 27–30, *l*=1–3 series, cream, translucid. **Stipe** 5–10 × 1mm, central, cylindrical, fibrillose, dry, pubescent, insititious, brownish to cream upward. Many brown, filiform rhizomorph accompanied the basidiomes.

Basidiospores 5.8–8.5 × 2.8–4.0 μm [χ_m= 7.2 ± 1.0 × 3.4 ± 0.4 μm; Q_m= 2.1 ±0.3 μm; n=6], ellipsoid to lacrimoid, thin-walled, inamyloid, hyaline. **Basidia** 21 × 5 μm, clavate, 4-sterigmate, thin-walled, hyaline. **Basidioles** 18–20 × 5–5.5 μm, clavate, thin-walled, hyaline. **Cheilocystidia** main body 24–30 × 6–8 μm, clavate, fusiform, thin-walled, hyaline. **Pleurocystidia** absent. **Pileipellis** irregular, prostrated, incrusted, brownish cells with terminal cells, clavate, brownish, incrustated, some fusiform, hyaline. **Pileus trama** interwoven. **Lamellar trama** similar to pileus trama. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: gregarious, leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 25 April 2013, D.L.Komura & P.A.Pereira, DLK1346 (INPA259642), DLK1352 (INPA259643), DLK1380 (INPA259659).

Comments: This specimen is very similar to *Gymnopus* sp.9, but with rhizomorphs (telepods). The sequences of this specimen presented many ambiguous bases (DLK1352), and it was unable to use in the analysis. So we could not confirm if have or not differences in the molecular level.

16- *Rhodocollybia* sp. 1

Figure 17

Pileus 16–20 mm diam, broadly convex to plan, some slightly depressed or slightly umbonate margin striate, uplift, incurved, discolorous, hygrophanous, shiny cream to pale rose (EDCDAB) to center brownish (A85700) and margin whitish; **context** thin, cream. **Lamellae** adnate, subdistant, *L*= 9–11, *l*= 6–8 series, smooth, membranaceous, concolorous with pileus. **Stipe** 15–20 × 3 mm, central, cylindrical, hollow, hygrophanous, translucid, smooth, pale cream, with bulbous base.

Basidiospores 6 × 4 μm, most of the spores was imature, lacrimoid to ellipsoid, smooth, thin-walled, hyaline. **Basidia** 20 × 5 μm, clavate, 4-sterigmate, thin-walled, hyaline. **Basidioles** 20 × 5 μm, clavate, thin-walled, hyalin. **Cheilocystidia** not observed. **Pleurocystidia** absent. **Pileipellis** formed of prostate hyphae, parallel, irregular, brownish, thin-walled. **Pileocystidia** not observed. **Pileus trama** 5–12 μm diam, two hyphae, one thin, smooth, and another inflate, thin-walled, with brownish glutinous. **Lamellar trama** 4 μm diam, interwoven, thin-walled, hyaline, inamyloid, **Stipitipellis** 4–6 μm diam, parallel cells, smooth, thin-walled, hyaline. **Caulocystidia** 5–6 × 3 μm, finger-like projections, thin-walled, hyalin. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: solitary and caespitose, decay trunk and soil with organic material attached, at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Reserva de Desenvolvimento Sustentável do Tupé, 05 Jun. 2012, D.L.Komura, DLK777 (INPAXXXX), 07 Jun 2012, D.L.Komura DLK803; Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 May 2012, F. C. Batista & D. L. Komura, DLK714 (INPAXXX), DLK724 (INPAXXX); 28 Jun. 2012, D.L.Komura, DLK843 (INPAXXX); 23 May 2013, O. F. Menezes & D. L. Komura, DLK1499 (INPA259662).

Comments: The ITS sequences from this specimen were placed in the same clade with high support (100% BS). Just one sequence (DLK1499) was divergent in the same clade. No significant morphological differences were observed among the specimens. Our species have similar pileus description from *R. dotae* (Mata et al. 2004), but is completely different for other features, specilly among the number of lamellae, most species in *Rhodocollybia* has

close to crowded lamellae, while our is distant. A more accurate microscopic analysis is needed to confirm if this taxon belong to *Rhodocollybia*.

17- *Marasmiellus* sp. 1

Figure 18

Pileus 5–12 mm diam, conchiform to flabeliform, subvelutinous, brownish pale (A68671); **context** whitish, thin. **Lamellae** adnate, distant, *L*= 3–4, thin, smooth, cream, reticulate, forked, unequal. **Stipe** absent or extremely short, at maximum 1 mm long, lateral, cylindrical.
Basidiospores 7.0–9.0 × 4.0–5.5 µm [χ_m= 8.1 ± 0.7 × 4.9 ± 0.4 µm; Q_m= 1.7 ±0.2 µm; n=15], elliptical to lacrimoid, thin-walled, inamyloid, hyaline. **Basidia** 25-30 × 6-8µm, clavate, 4-sterigmate. **Basidioles** 22–30 × 5–8 µm, clavate, some fusoid, thin-walled. **Cheilocystidia** not observed. **Pleurocystidia** not observed. **Pileipellis** irregular, agglutinated, with *Rameales*-like terminal cells, elongate, lobate, knobby diverticula. **Pileus trama** similar to lamellar trama. **Lamellar trama** parallel to interwoven. **Clamp connections** present in all tissues.
Habit, substrate and known distribution: pleurotoid, gregarious on branch at secondary terra firme forest.
Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1315 (INPA271948).

18- *Marasmiellus* "white4"

Figures 19 a and b

Pileus 3–8 mm diam, convex to broadly convex, velutinous, dull, fragile, whitish turning brownish pale (CCAE85) in age, brown scale-like hairs on the pileus under lens; **context** whitish, thin. **Lamellae** adnate, distant, *L*= 6–9, thin, smooth, cream, slightly reticulate, forked, unequal. **Stipe** central, cylindrical, white, turning copper brownish in age, shinny, with fibrillose white cover, insititious on substrate.
Basidiospores 12.0–13.0 × 4.0–5.0 µm [χ_m= 12.5 ± 0.5 × 4.6 ± 0.5 µm; Q_m= 2.8 ±0.3 µm; n=8], elliptical to amygdaliform, thin-walled, inamyloid, hyaline. **Basidia** 25–30 × 6–8 µm, clavate, 4-sterigmate. **Basidioles** 25–35 × 5–7 µm, clavate, thin-walled. **Cheilocystidia** not observed. **Pleurocystidia** 65 µm long, cylindric to afilate, then slightly capitulate, obclavate, thin-walled, hyaline. **Pileipellis** irregular, agglutinated, cells. **Pileus hairs** scanty, elongate, some, cylindrical, apex obtuse, filiform, fusiform, setiform, some very branched with acute apex, thick-walled, brown to golden-brown. **Pileus trama** similar to lamellar trama. **Lamellar trama** parallel to interwoven. **Stipitipellis** parallel, slightly thick-walled, brownish,

cell 4 –10 μm diam; termnial cell elongate, apex obtuse. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: gregarious on branch at primary and secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 27 Jun. 2012, D.L.Komura, DLK824 (INPAXXXX); RDS Tupé, 24 Mar. 2013, D. Cardoso & D.L. Komura, DLK1064 (INPAXXX); 10 Sep. 2013, D.L.Komura & J.R. Maciel, DLK1720 (INPA259513); DLK1721 (INPA259514).

Comments: The three sequences are placed together with high support (100%).

19- *Marasmiellus* "brown4"

Figure 20

Pileus 5–10 mm diam, broadly, radially fibrillose, opaque, dark brown (4F3817) at center to brownish (9C805A); **context** brownish, thin. **Lamellae** adnate, distant, *L*= 7–10, *l*= 1 series, intervenose, bifurcate, thin, smooth, pale brown. **Stipe** 1 × 3–7 mm, central, cylindrical, insititious, dark brown to brown upward.

Basidiospores 10.0–12.0 × 5.0–6.0 μm [χ_m= 11.0 ± 0.9 × 5.7 ± 0.4 μm; Q_m= 1.9 ±0.2 μm; n=12], elliptic to fusiform, thin-walled, hyaline. **Basidia** 30-35 × 9 μm, clavate, 2-sterigmate. **Basidioles** 28–35 × 6–9 μm, clavate, fusiform, thin-walled. **Cheilocystidia** not observed. **Pleurocystidia** not observed. **Pileipellis** parallel hyphae, inflated, around 15 μm diam, and thinner, 5 μm diam, thin-walled, hyaline, with hair-like cells, elongate, think-walled, brown, clamped,

Habit, substrate and known distribution: gregarious on branch at primary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Jun. 2012, D.L.Komura, DLK819 (INPA259713); 24 Apr 2013, D.L.Komura & P.A. Pereira, DLK1268 (INPA259708).

20- *Marasmiellus* sp. 8

Figures 21a and b

Pileus 6–9 mm diam, convex, brown (9E8875), radially fibrillose, felted; **context** thin, cream. **Lamellae** adnate, subdistant, *L*= 14, *l*= 1–2 series, cream. Stipe 1 × 7 mm, cylindrical, fibrous, greyish.

Basidiospores not observed. **Basidia** not observed. **Basidioles** 25 × 5μm, clavate, hyaline. **Cheilocystidia** absent. **Pleurocystidia** absent. **Pileipellis** irregular, prostrated and inflated

cells, incrustrated. **Pileocystidia** hair-like, long (> 100 × 4μm), thin-walled, hyaline and subclavate, irregular, cylindrical, inflated, thick-walled, brownish, incrustrate cells. **Pileus trama** interwoven, thin and incrusted cells. **Lamellar trama** interwoven, thin-walled.

Habit, substrate and known distribution: marasmioid, solitary at branch on the leaf litter at secondary terra firme forest.

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK448 (INPA259650).

Comments: This specimen is immature, any basidia, neither basidiospores were observed. The ITS sequence are collapsed among *Marasmiellus* group.

21- ***Marasmiellus cubensis*** (Berk. & M.A. Curtis) Singer, Sydowia 15 (1-6): 57 (1961) [MB#333631]

Holotype: K(M). **Type locality:** Cuba.

Figure 22

Pileus 10–15 mm diam, convex to broadly convex, sulcate margin incurved, smooth, opaque, pure white. In herbarium turning brown to dark brown, except the mycelia under the substrate, which keep white; **context** white, thin. **Lamellae** adnate, subdistant, *L*= 15–17, *l*= 0–1 series, thin, smooth, white intervenose. **Stipe** 6–10 × 2 mm, central, cylindrical, glabrous, shiny, rising from whitish mass of mycelial disc.

Basidiospores 10.6–13.0 × 5.0–6.0 μm [χ_m= 11.9 ± 0.9 × 5.7 ± 0.5 μm; Q_m= 2.1 ±0.2 μm; n=7] ellipitical to lacrimoid, thin-walled, inamyloid, hyaline. **Basidia** 40 × 7 μm, clavate, 4-sterigmate. **Basidioles** 35–40 × 6–8 μm, clavate, some fusoid, with drops. **Cheilocystidia** absent. **Pleurocystidia** 50 × 5 μm, elongate, cylindrical, fusiform. **Pileipellis** 4–6 μm diam, one type, thinner, interwoven, acute terminal cells. Another thicker, brownish. **Pileus trama** interwoven. **Lamellar trama** parallel to interwoven. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: gregarious on branch and trunk on the leaf litter, at secondary forest and campinarana forest. Brazil, Trinidad, Cuba (Dennis 1951, Singer 1973).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 10 May 2012, D.L.Komura & T.S. Marinho, DLK456 (INPAXXX); 08 Fev. 2013, D.L.Komura & J.R.Maciel, DLK1719 (INPA259718); Roraima State, Caracaraí, Parque Nacional do Viruá, D.L.Komura & P.A.Pereira, DLK1050 (INPA271943).

Comments: The description in Dennis [as *Collybia cubensis* (Berk. & Curt.) Dennis 1951] are similar to our specimen. This species is similar to *M. volvatus*, but lack a volva-like

structure in the base of the stipe and also in herbarium, this species turning dark brown. The ITS sequences also split these species with high support (100% BS) for each species.

22- ***Marasmiellus volvatus*** Singer, Fieldiana Botany 21: 37 (1989)

[MB#124903]

Holotype: INPA. **Type locality:** Amazonas, Brazil.

Figure 23

Pileus 5–20 mm diam, convex in immature basidiome, turning broadly convex in age, sulcate, margin incurved, smooth, opaque when young turning translucent in age, hygrophanous, pure white. In herbarium, turning cream; **context** white, thin. **Lamellae** adnate, subdistant, *L*= 11–12, *l*= 1 series, thin, smooth, white. **Stipe** 5–12 × 2 mm, central, cylindrical, glabrous, softly, insititious, bulbous base with volva-like cover.

Basidiospores 15.0–17.0 × 5.0–6.0 µm [χ_m= 16.0 ± 0.9 × 5.3 ± 0.4 µm; Q_m= 3.1 ±0.2 µm; n=6], fusoid. **Basidia** 35–37 × 10 µm, clavate, 4-sterigmate. **Basidioles** 30–40 × 5-10 µm, clavate, thin-walled. **Cheilocystidia** absent. **Pleurocystidia** absent **Pileipellis** irregular, agglutinated. **Pileocystidia** main body 20 × 7 µm, clavate, pyriform, diverticulate, branched, lobate, irregular. **Pileus trama** similar to lamellar trama. **Lamellar trama** parallel to interwoven. **Stipitipellis** parallel, hyaline. **Caulocystidia** absent. **Clamp connections** present in all tissues.

Habit, substrate and known distribution: gregarious on branch at primary terra firme forest and branches at campinarana forest. This species of *Marasmiellus* appears to be widely dispersed in the lowland Neotropics, Costa Rica (Halling 2003), Brazil (Singer 1989).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 08 May 2012, D.L.Komura & T.S. Marinho, DLK417 (INPA271932); 23 May 2013, D.L.Komura & O.F.Menezes, DLK1502 (INPA259716); 23 Jun. 2013, D.L.Komura, M.R.Pereira, D.S.Ferreira & L.S.Bento, DLK1665 (INPA259717); São Gabriel da Cachoeira, Comunidade Itacoatiara Mirim, 05 April 2013, D.L.Komura & D. Cardoso, DLK1134 (INPA259714).

Comments: This species was described by Singer (1989) from Manaus, Amazonas, Brazil. It can be distinguished by the whitish, plicate pileus, insititious and bulbous stipe with a fine volva remnant. Sometimes is similar to *M. cubensis*, but this species just present a mycelial mass at the stipe base and also in herbarium *M. volvatus* keeping a pale color, while *M. cubensis* turning dark brown.

23- ***Marasmiellus ramealis*** **var.** ***tucumanensis*** Singer, Beihefte zur Nova Hedwigia 44: 331 (1973)

[MB#348398]

Holotype: F. **Type locality:** Tucumán, Argentina.

Figure 24

Pileus 5 mm diam, broadly convex, sulcate, margin incurved, smooth, opaque, hygrophanous, white to cream, turning red ferrugineous when injured; **context** white, thin. **Lamellae** adnate, distant, L= 8-9, l= 3 series, thin, smooth, conolorous with pileus. **Stipe** 2–3 × 0.5 mm, very short, eccentric, incurved, cylindrical, glabrous, softly, slightly shiny, insititious.

Basidiospores 7.0–9.0 × 5.0–6.0 μm [χ_m= 8.3 ± 0.7 × 5.7 ± 0.5 μm; Q_m= 1.5 ±0.1 μm; n=20], elliptic to lacrimoid, thin-walled, amyloid, hyaline. **Basidia** 24–26 × 6 μm, clavate, 2-, 4-sterigmate. **Basidioles** 20–25 × 5 μm, clavate, thin-walled. **Cheilocystidia** similar to pileocystidia. **Pleurocystidia** absent **Pileipellis** irregular, agglutinated. **Pileocystidia** main body 10–16 × 5–10 μm, cylindric to subclavate with diverticula shorter than usual, clavate, pyriform, some bifurcate, thin-walled, hyaline. **Pileus trama** interwoven, inamyloid. **Lamellar trama** interwoven, thin-walled, hyaline, subhymenium dextrinoid. **Stipitipellis** parallel, hyaline, clamped. **Caulocystidia** absent.

Habit, substrate and known distribution: gregarious on branch and leaf on the leaf litter, at primary and secondary terra firme forest. *Marasmius ramealis* was described to temperate and subtropical area. This variety was described to Argentina (Singer 1973).

Material examined: Brazil, Amazonas State, Manaus, Estação Experimental de Manejo Florestal ZF-2, 24 Apr. 2013, D.L.Komura & P.A. Pereira, DLK1328 (INPA259620) 23 May 2013, D.L.Komura & O.F.Menezes, DLK1501 (INPA259621); Roraima State, Caracaraí, Parque Nacional do Viruá, 08 Aug. 2013, D.L.Komura & P.A. Pereira, DLK1048 (INPAXXXX).

Comments: Our specimen is very similar with description in Singer (1973) with *M. ramealis* var. *tucumanensis*, the cheilocystidia descriptions are identical, with clavarioid cells with diverticules, just the basidiospores are narrow than we found in our samples (2.5- 3.2 μm *vs* 5.0-6.0 μm). The sequences from Amazonas State (DLK1501) and Roraima State (DLK1048) are grouped with high support (1005 BS) in the phylogenetic tree, the morphological characters also is similar for both samples.

Conclusions

The ITS sequences was a usefull marker to species delimitation associated with morphological characters. However, a caution on the alignment step is indispensable once that ITS sequence is a very variable region. For purpose to understand the evolutionary relationship within the Omphalotaceae group, multigene analysis, accurate descriptions for the specimens, study the type for each section, is necessary for a comprehensive understand of this group.

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Legends

Table S1. Strains and GenBank accessions of ITS sequences used in this study.

Figure 1. Molecular Phylogenetic analysis by Maximum Likelihood method from Omphalotaceae spp. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The taxa with description are marked with colored boxes around the taxa name.

Figure 2. *Gymnopus* aff. *parvulus*. A- basidiomata; B, E- lamellae; C, F- pileus surface; D- basidiomata lateral view and superficial hyphae on the leaf (shiny and whitish) Scale bar 10 mm.

Figure 3. *Gymnopus* sp.16. A, B- basidiomata. C- lamellae view; D- detail of the mycelial pad from stipe. Scale bar 3.5 mm.

Figure 4. *Gymnopus* sp. 2. A- basidiomata; B- hymenium. Scale bar= 3.5 mm.

Figure 5. *Gumnopus* sp.1. A, G- basidiomata; C, E- pileus sufae; D, F- lamellae; B- mycelial pad on the sitpe base. Scale bar 15 mm.

Figure 6a. *Gymnopus* sp.3. A- basidiomata; B- pileus surface; C- hymenium color in age; C- hymenium young specimen.

Figure 6b. Microscopic features of *Gymnopus* sp.3. A- basidiospores; B- hymenial layer, basidia with basdiospores; C- hymenial layer in congo red, showing basdiospores; D- basidia and basidioles; E- cheilocystidia; F- stipetipelis.

Figure 7. *Gymnpous* DLK1852. A- basidiomata; B, C- lamellae; D- pileus surface. Scale bar 25 mm.

Figure 8a. *Gymnopus* sp.13. A- basidiomata; B- lamellae. Scale bar= 5mm.

Figure 8b. Microscopic features of *Gymnopus* sp.13. A- basidiospores; B basidia and basidioles; C- cheilocystidia; D- pileocystidia; pileus trama hyphae. Scale bar 10 μm. Illustrated by D.L.Komura.

Figure 9. *Gymnopus* sp.30. A- basidiomata; B- lamellae. Scale bar 10 mm.

Figure 10a. *Gymnopus* sp. 12- A, C- young basidiomata; B, F- lamellae; D,E- pileus suface. Scale bar a, f= 3.5; c= 2.5.

Figure 10b. Microscopic features of *Gymnopus* sp.12. A- basidiospores; B- basidia and basidioles; C- cheilocystidia; D- pileipellis hyphae; E- stipetipellis hyphae. Scale bar= 10 μm. Illustrated by D.L.Komura.

Figure 11. *Gymnopus* sp.6. A- lamellar features; B- basidiomes.Scale bar 5 mm.

Figure 12a. *Gymnopus* sp.4. A- basidiomes; B, C- pileus surface; D, E- lamellar features. Scale bar= 5 mm.

Figure 12b. Microscopic features of *Gymnopus* sp. 4, Microscopic features. A- lamellar trama and cheilocystidia; B- basidospores; C- basidia and basidioles in congo red; D- lamellar trama; E and F- cheilocystidia. Scale bar: B= 5 μm; A, D, E= 20 μm; D, F= 10 μm

Figure 13a. *Gymnopus* sp.14. A- basidiomata; B, D-lamellae view; C, E pileus surface. Scale bar 10 mm.

Figure 13b. Microscopic features of *Gymnopus* sp.14. A- basidiospores; B- basidioles and badisidium; C- cheilocystidia; D- pileipellis hyphae; E-stipetipellis elements. Scale bar 10 μm. Illustrated by D.L.Komura.

Figure 14a. *Gymnopus* sp. 17. A- basidiomata; B, D- pileus surface; C, E- lamellae view. Scale bar 10 mm.

Figure 14b. Microscopic features of *Gymnopus* sp.17. A- basidiospores; B- basidioles; C- cheilocystidia; D- pileipellis hyphae; E-stipetipellis elements. Scale bar 10 μm. Illustrated by D.L.Komura.

Figure 15. *Gymnopus* sp.9. A- basidiomata on the leaf; B- lateral view, C- young basidiomata; D- detail of lamellae; E- pileus surface. Scale bar 15 m.

Figure 16a. *Gymnopus* sp.20. A- basidiomata, young specimen; B- detail of the young specimen and telepods; C, D- lamellae; E- pileus surface. Scale bar A, C = 10 mm.

Figure 16b. Microscopic features of *Gymnopus* sp.20. A- sagittal cutting, showing pileipellis; B- lamellar trama; C- detail of lamellar edge with cehilocystidia; D- basidium; E, F- pileipellis. Scale bar 60 μm.

Figure 17. *Rodocollybia* sp.1. A- detail of hymenium; B- pileus; C- basidiomatas. Scale bar 10 mm.

Figure 18. *Marasmiellus* (DLK1315). A- Basidiomata; B- detail of the hymenium. C- Pileipellis; D- Basidiopores; E- Basidioles.

Figure 19a. *Marasmiellus* "white4". A- Basidiomatas; B,D- lamellar view; C, E- older basidiomatas. Scale bar 10 μm.

Figure 19b. Microscopic features of *Marasmiellus* "white4". A- lamellar edge; B-basidia; C- pleurocystidia; D, F, H- pileocystidia; E,G- stipetipellis. Scale bar 10 μm.

Figure 20. *Marasmiellus* "brown3". A, B- basidiomata; C-basidiospores; D, E- Pileipellis.

Figure 21a. *Gymnopus* sp.8. A-basidiomata, detail of insititious stipe; B- pileus surface; C- lamellae; D- basidiomata. Scale bar 3mm.

Figure 21b. Microscopic features of *Gymnopus* sp.8. A, C-lamellar trama; B,D- pileipellis, incrusted hyphae. Scale bar: A= 40 μm.

Figure 22. Macroscopic and microscopis features of *Marasmiellus cubensis*. A- basidiomata; B- lamellae; C- detail of the stipe base; D- basidia with basidiospores; E, G- lamellar trama; F- pileipellis. Scale bar A= 15 mm.

Figure 23. *Marasmiellus volvatus*. A- basidiomata; B- lamellae; C- basidiomata, young specimen; D- lamellae, young specimen; E, F- detail of the volva-like stipe base; G- pileus surface. Scale bar 10 mm.

Figure 24. *Marasmiellus* "cream1". A, C- basidiomata; B, D- lamellar face.

Table S1. Strains and GenBank accessions of ITS sequences used in this study

Species	collection ID	Herbarium accession No.	Location	Genbank accession No.	References
Gymnopus parvulus		TENN58119	Costa Rica	DQ450062	Mata et al. 2007
Gymnopus parvulus	TFB10419		Costa Rica	NR119584	Mata et al. 2007
Gymnopus parvulus	FB10422		Costa Rica	AF505774	Mata et al. 2007
G. aff. *parvulus*	DLK1287	INPA259618	AM, Brazil		this work
G. aff. *parvulus*	DLK1284	INPA259617	AM, Brazil		this work
G. aff. *parvulus*	DLK1289	INPA259619	AM, Brazil		this work
Gymnopus	DLK1931	INPA271972	AM, Brazil		this work
Gymnopus sp.16	DLK707	INPA259692	AM, Brazil		this work
G. collybioides		FB100080	Costa Rica	AF505772	Mata et al. 2007
G. biformis		TENN58088	Costa Rica	DQ450055	Mata et al. 2007
G. biformis		TENN58624	Costa Rica	DQ450056	Mata et al. 2007
G. cylindricus	100084		Costa Rica	AY256696	Mata et al. 2007
G. cylindricus	TFB10091	TENN58024	Costa Rica	DQ450057	Mata et al. 2007
G. cylindricus	FB10402		Costa Rica	AF505776	Mata et al. 2007
G. biformis		TENN58024	Costa Rica	DQ450064	Mata et al. 2007
G. alnicola	REH8266		Costa Rica	AF505770	Mata et al. 2007
G. mesoamericanus	TFB11005		Costa Rica	NR119583	Mata et al. 2007
G. neotropicus	TFB10416		Costa Rica	AF505769	Mata et al. 2007
G. menehune	AWW87			AY263444	Wilson et al. 2003
G. subcyathiformis		TENN55243	Mexico	DQ450037	Mata et al. 2007
G. subcyathiformis		TENN58642	Costa Rica	DQ450038	Mata et al. 2007
G. subcyathiformis		TENN58130	Costa Rica	DQ450040	Mata et al. 2007
Gymnopus sp.2	DLK1474	INPA259661	AM, Brazil		this work
Gymnopus sp.1	DLK376	INPA271930	AM, Brazil		this work
Gymnopus sp.1	DLK511	INPA259710	AM, Brazil		this work
G. fibrosipes	FB9699		Costa Rica	AF505763	Mata et al. 2007
G. fibrosipes	PR23TN		Puerto rico	AY842953	Lopez; Rios-Velazquez 2004
G. luxurians	FB10350		USA	AF505765	Mata et al. 2007
Gymnopus	DLK1852	INPA271968	PA, Brazil		this work
Gymnopus sp.3	DLK1311	INPA259654	AM, Brazil		this work
Gymnopus sp.3	DLK1334	INPA259658	AM, Brazil		this work
Gymnopus sp.13	DLK1386	INPA259683	AM, Brazil		this work
Gymnopus sp.30	DLK621		AM, Brazil		this work
Gymnopus sp.12	DLK1303	INPA259678	AM, Brazil		this work
Gymnopus sp.12	DLK611	INPA259671	AM, Brazil		this work
Gymnopus sp.12	DLK1198	INPA259675	AM, Brazil		this work
Gymnopus sp.12	DLK1313	INPA259679	AM, Brazil		this work
Gymnopus sp.12	DLK1069		AM, Brazil		this work
Gymnopus sp.12	DLK1345	INPA259681	AM, Brazil		this work
Gymnopus sp.6	DLK1388	INPA259645	AM, Brazil		this work
Gymnopus sp.B	DLK725	INPA259616	AM, Brazil		this work
Gymnopus sp.4	DLK1646	INPA259665	AM, Brazil		this work
Gymnopus sp.4	DLK1385		AM, Brazil		this work
Gymnopus sp.4	DLK986		AM, Brazil		this work
G. omphaloides	FB11021		Costa Rica	AF505761	Mata et al. 2007
G. pseudolodgeae	FB10493		Costa Rica	AF505747	Mata et al. 2007
G. pseudolodgeae	FB10493		Costa Rica	NR119462	Mata et al. 2007
G. lodgeae	FB11013		Costa Rica	AF505757	Mata et al. 2007
Gymnopus sp.14	DLK761	INPA259672	AM, Brazil		this work
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Gymnopus sp.14	DLK491	INPA259670	AM, Brazil		this work
Gymnopus sp.14	DLK489	INPA259668	AM, Brazil		this work
Gymnopus sp.14	DLK1209	INPA259677	AM, Brazil		this work
Gymnopus sp.17	DLK1491	INPA271955	AM, Brazil		this work

Gymnopus sp.17	DLK1694	INPA259699	AM, Brazil		this work
Gymnopus sp.17	DLK842	INPA271940	AM, Brazil		this work
Gymnopus sp.17	DLK1365	INPA259697	AM, Brazil		this work
Gymnopus sp.17	DLK695	INPA259690	AM, Brazil		this work
Gymnopus sp.9	DLK1400	INPA271952	AM, Brazil		this work
Gymnopus sp.9	DLK1757	INPA271960	AM, Brazil		this work
G. impudicus	FB9697		Costa Rica	AF505779	Mata et al. 2007
Micromphale sp.1	DLK1319	INPA259703	AM, Brazil		this work
Marasmius androsaceus			Sweden	DQ444316	Mata et al. 2006
Marasmius androsaceus			Sweden	JN943605	Fungal Barcoding Consortium
G. macropus	FB10095		Costa Rica	AF505788	Mata et al. 2007
G. spongiosus	FB11025		Costa Rica	AF505785	Mata et al. 2007
G. nubicola	REH8290		Costa Rica	AF505781	Mata et al. 2007
G. dryophilus	FB11015		Costa Rica	AF505787	Mata et al. 2007
R. prolixa var. *distorta*	EFM1403		Costa Rica	AF505748	Mata et al. 2007
R. pandipes		TENN53838	Costa Rica	AY313294	Mata et al. 2007
R. pandipes		TENN56641	Costa Rica	AY313295	Mata et al. 2007
R. cf. *pandipes*		TENN59546	Dominican Rep.	AY313295	Mata et al. 2007
Rhodocollybia sp.2	DLK812		AM, Brazil		this work
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R. dotae	REH7007		Costa Rica	AF505758	Mata et al. 2007
Rhodocollybia sp.1	DLK1499	INPA259662	AM, Brazil		this work
Rhodocollybia sp.1	DLK714		AM, Brazil		this work
Rhodocollybia sp.1	DLK724		AM, Brazil		this work
Rhodocollybia sp.1	DLK803		AM, Brazil		this work
Rhodocollybia sp.1	DLK843		AM, Brazil		this work
Omphalotus mexicanus		TENN51283	Mexico	AY313274	Mata et al. 2003
M. ramealis var. *tucumanensis*	DLK1048	INPA259390	RR, Brazil		this work
M. ramealis var. *tucumanensis*	DLK1501	INPA259621	AM, Brazil		this work
Marasmiellus white1	DLK606		AM, Brazil		this work
Tetrapyrgos brunneilucida	DLK1213	INPA259601	AM, Brazil		this work
Tetrapyrgos brunneilucida	DLK1339	INPA259594	AM, Brazil		this work
Marasmiellus white4	DLK1721	INPA259514	AM, Brazil		this work
Marasmiellus white4	DLK824		AM, Brazil		this work
Marasmiellus white4	DLK1720	INPA259513	AM, Brazil		this work
Marasmiellus sp.1	DLK1315	INPA271948	AM, Brazil		this work
Marasmiellus sp.C	DLK1439	INPA259704	AM, Brazil		this work
Marasmiellus brown4	DLK1268	INPA259708	AM, Brazil		this work
Marasmiellus brown4	DLK819	INPA259713	AM, Brazil		this work
M. volvatus	DLK1502	INPA259716	AM, Brazil		this work
M. volvatus	DLK417	INPA271932	AM, Brazil		this work
Marasmiellus sp.A	DLK1850		PA, Brazil		this work
Marasmiellus sp.B	DLK448	INPA259650	AM, Brazil		this work
M. cubensis	DLK1050	INPA271943	RR, BRAZIL		this work
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Figure 1 (part 1)

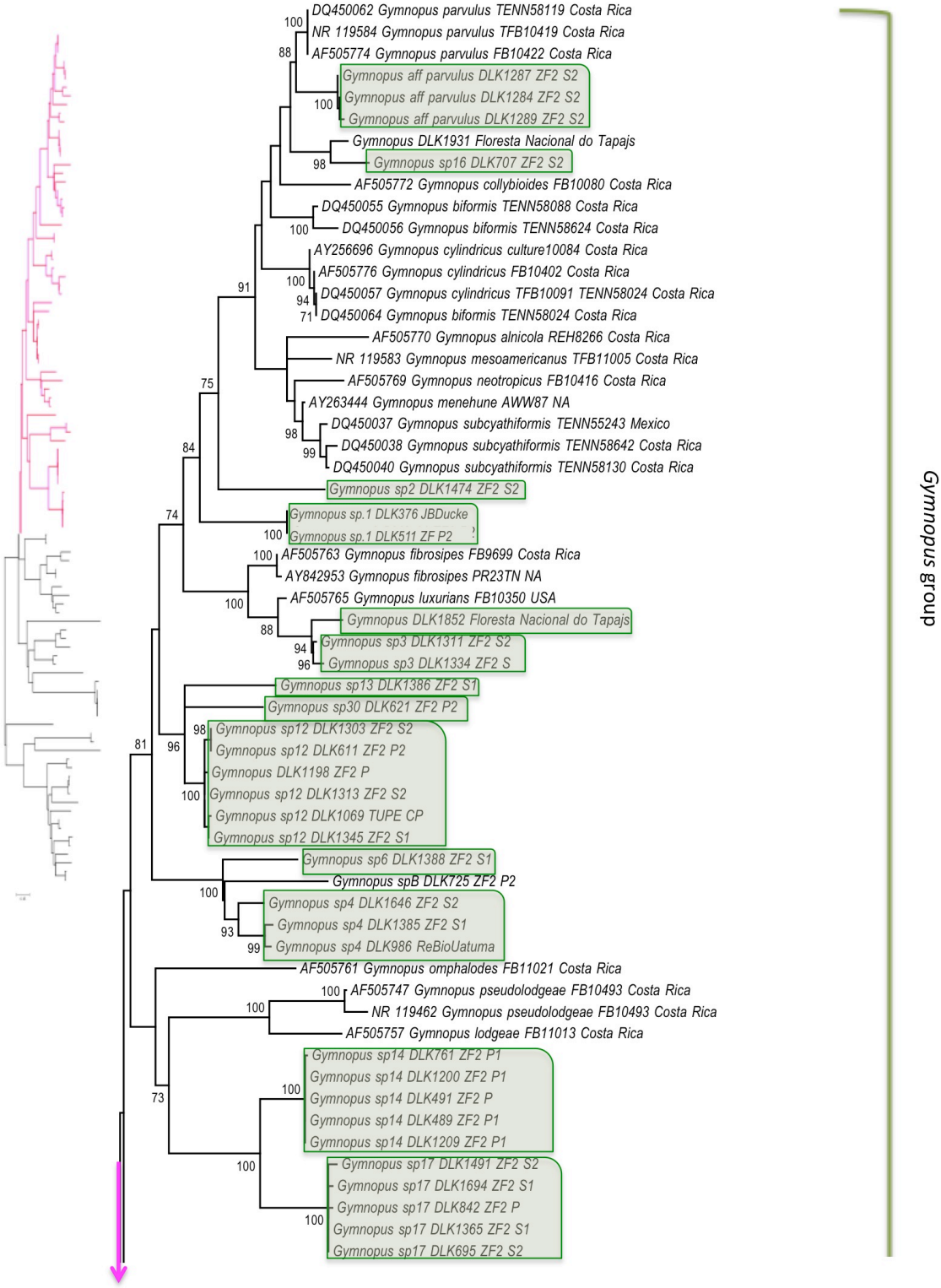


Figure1 (part 2)

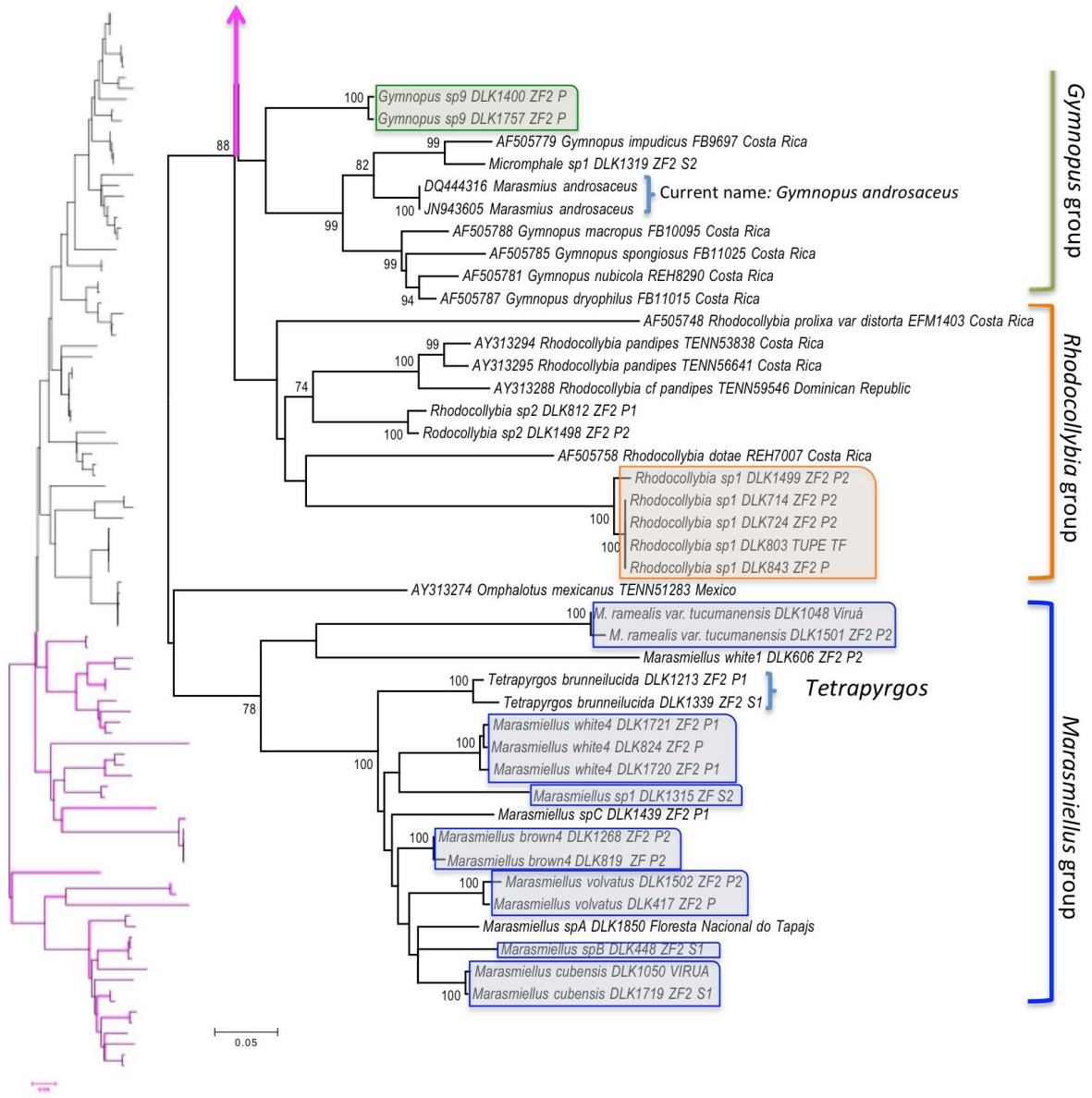


Figure 2- *Gymnopus* aff. *parvulus*

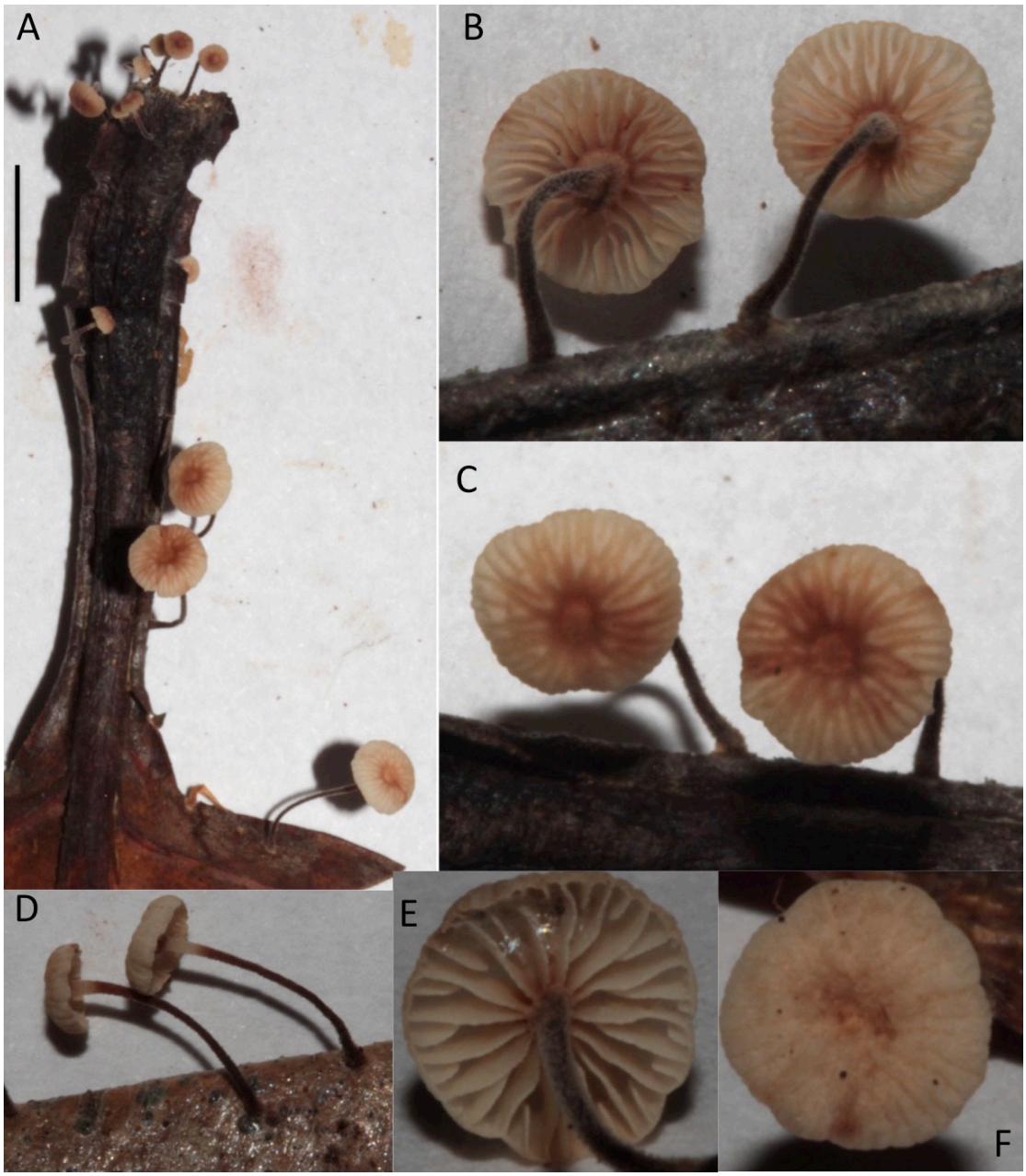


Figure 3- *Gymnopus* sp. 16

Figure 4- *Gymnopus* sp. 2

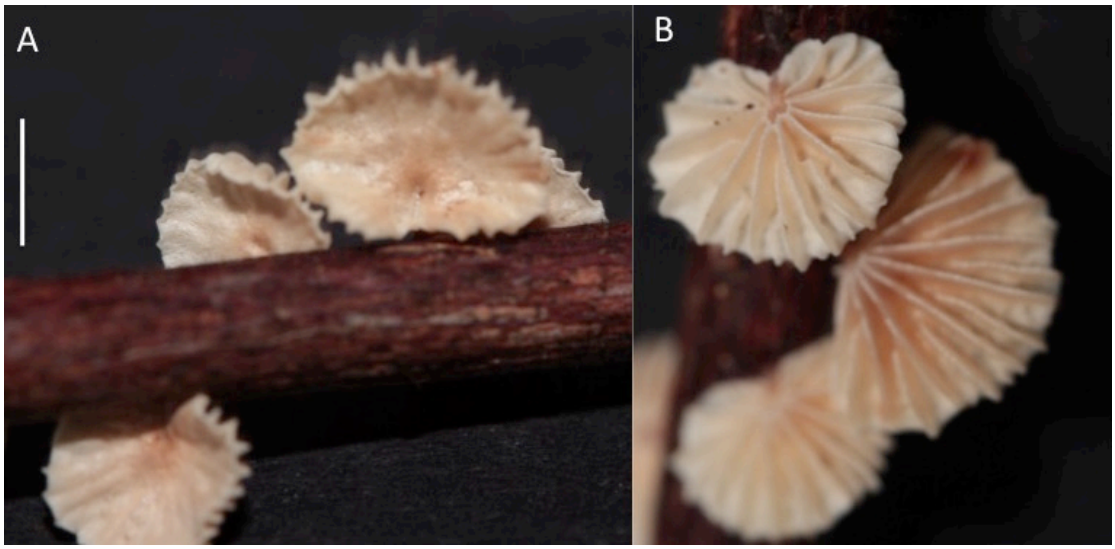


Figure 5- *Gymnopus* sp. 1

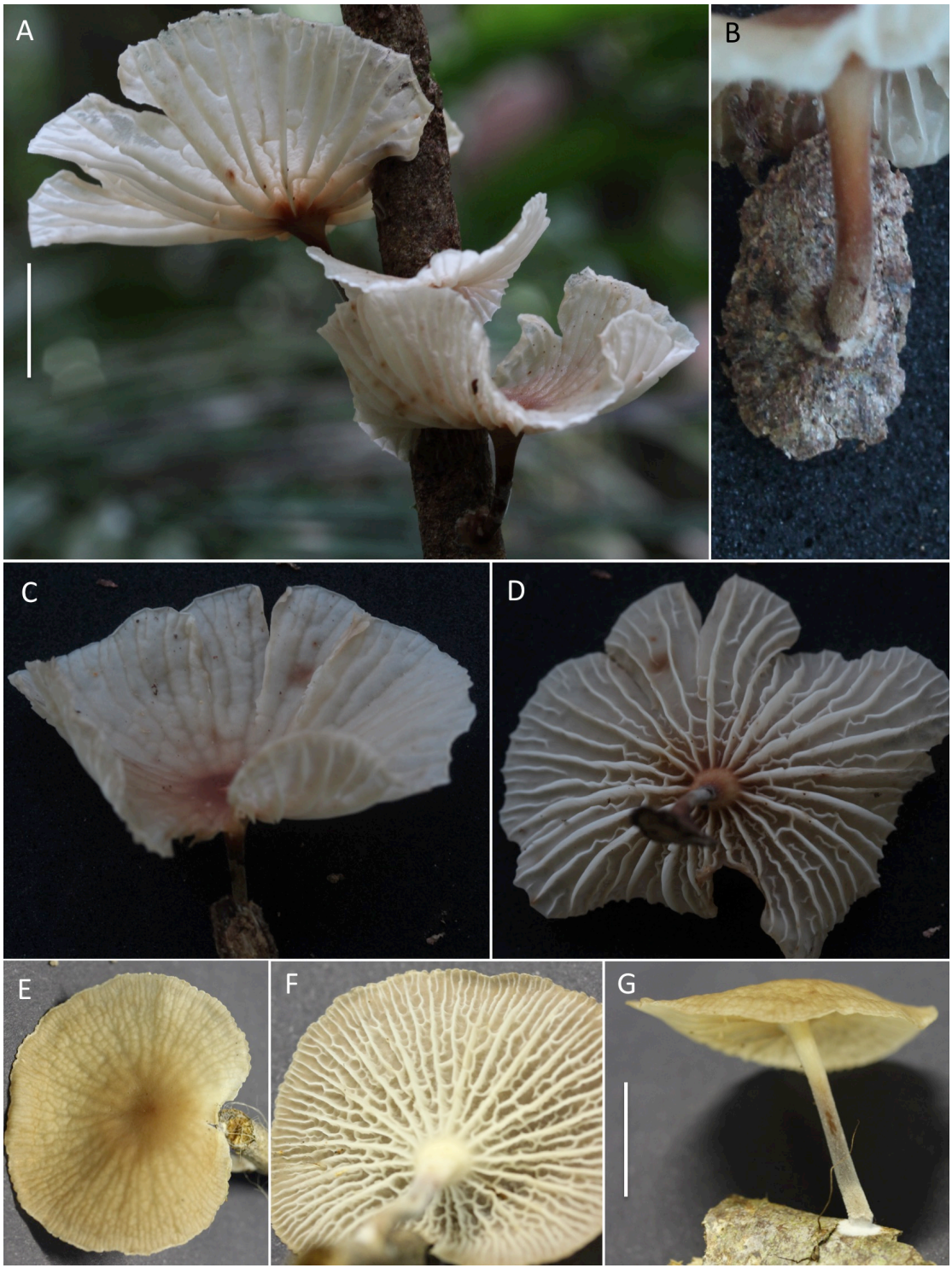


Figure 6a- *Gymnopus* sp. 3

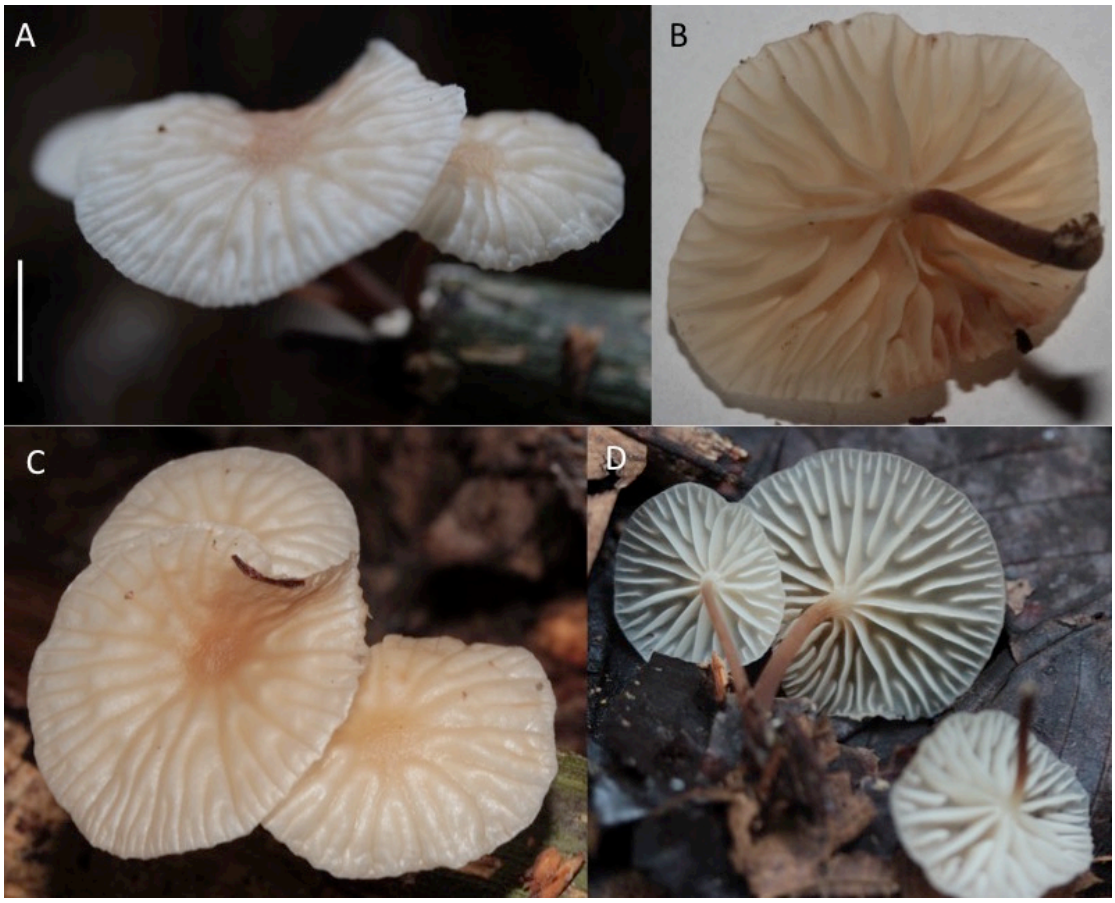


Figure 6b- *Gymnopus* sp. 3

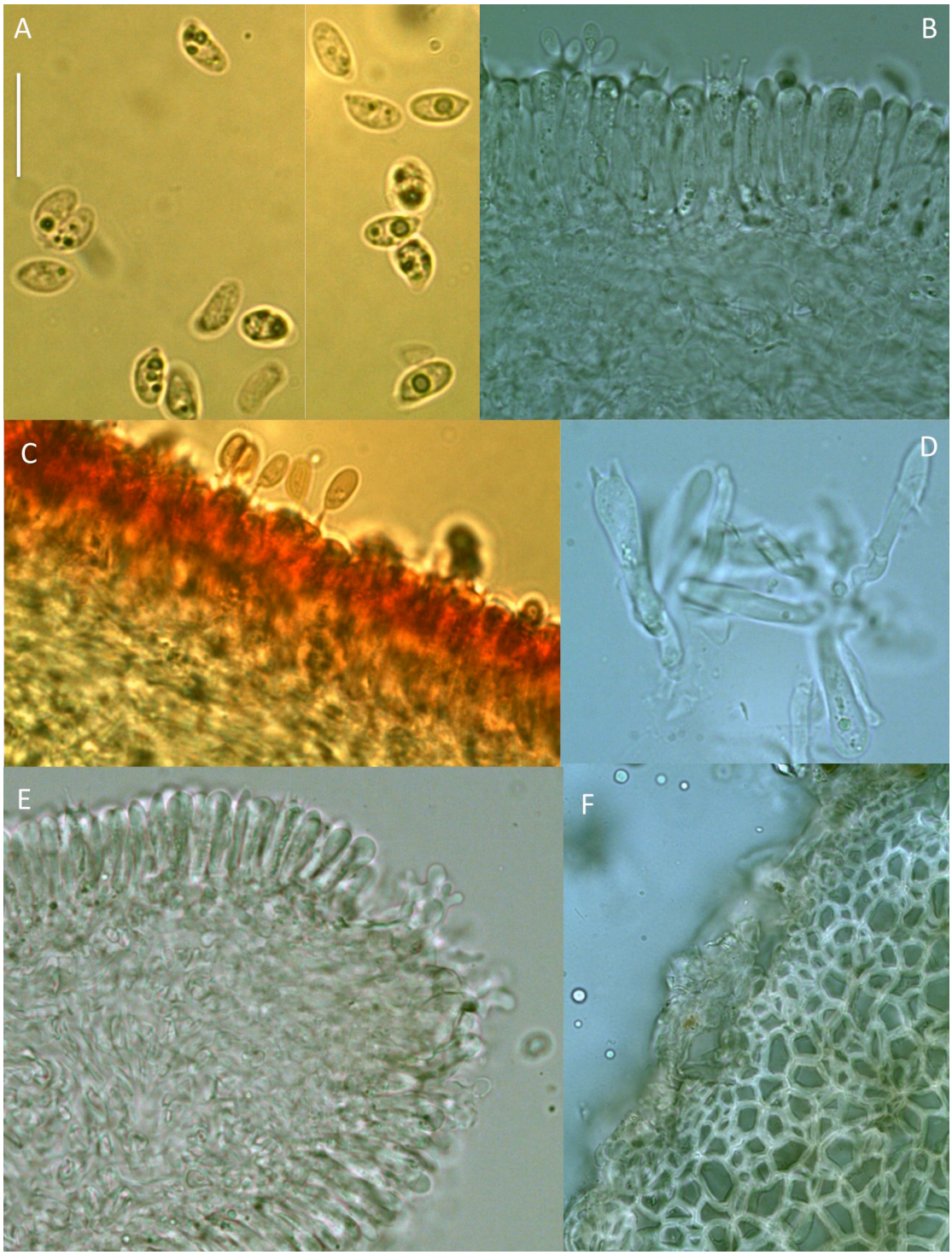


Figure 7- *Gymnopus* (DLK1852)

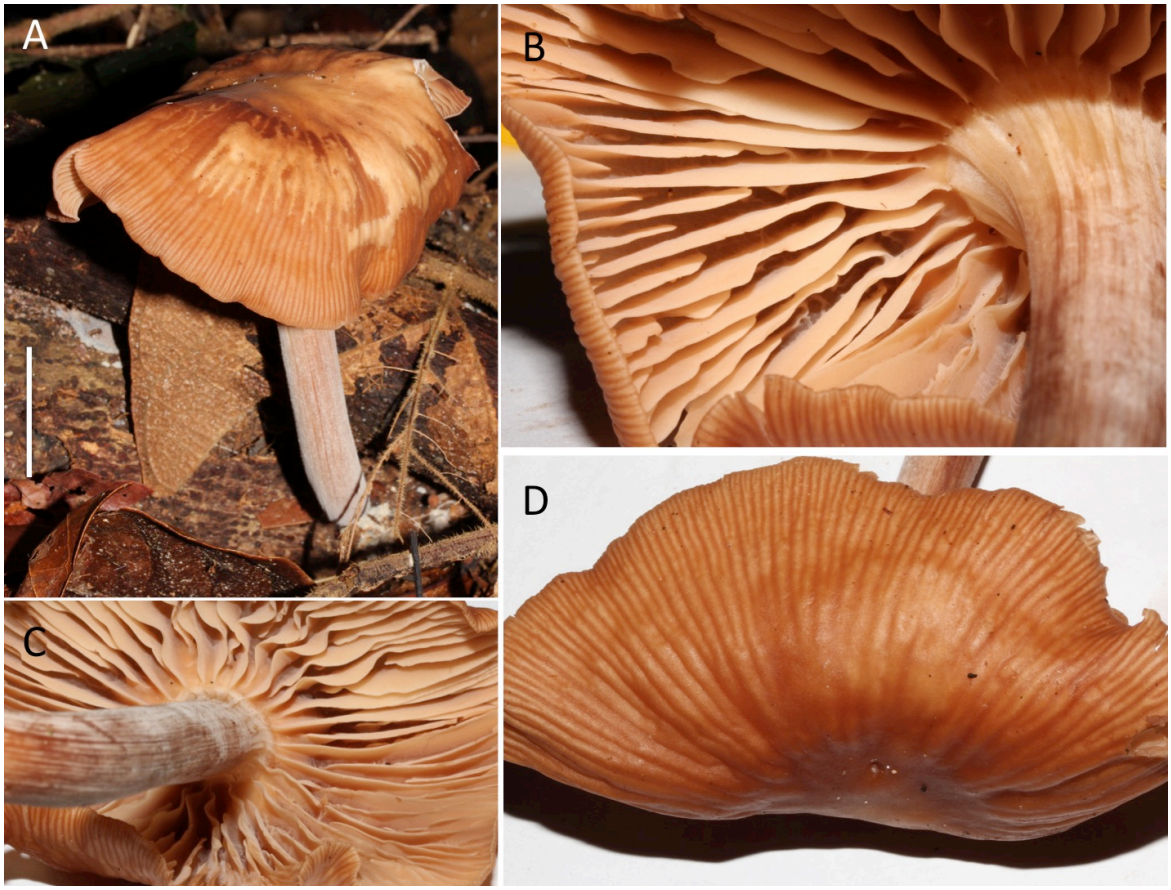


Figure 8a- *Gymnopus* sp. 13

Figure 8b- *Gymnopus* sp. 13

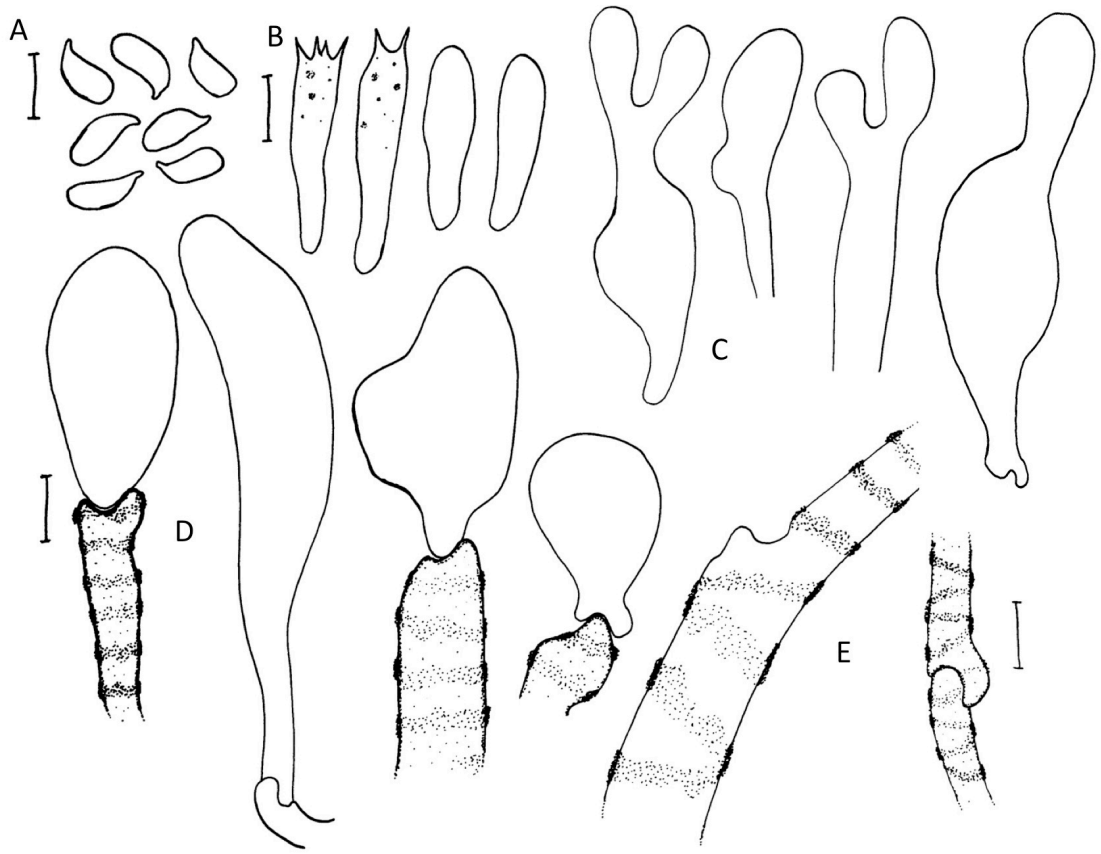


Figure 9- *Gymnopus* sp. 30

Figure 10a- *Gymnopus* sp. 12

Figure 10b- *Gymnopus* sp. 12

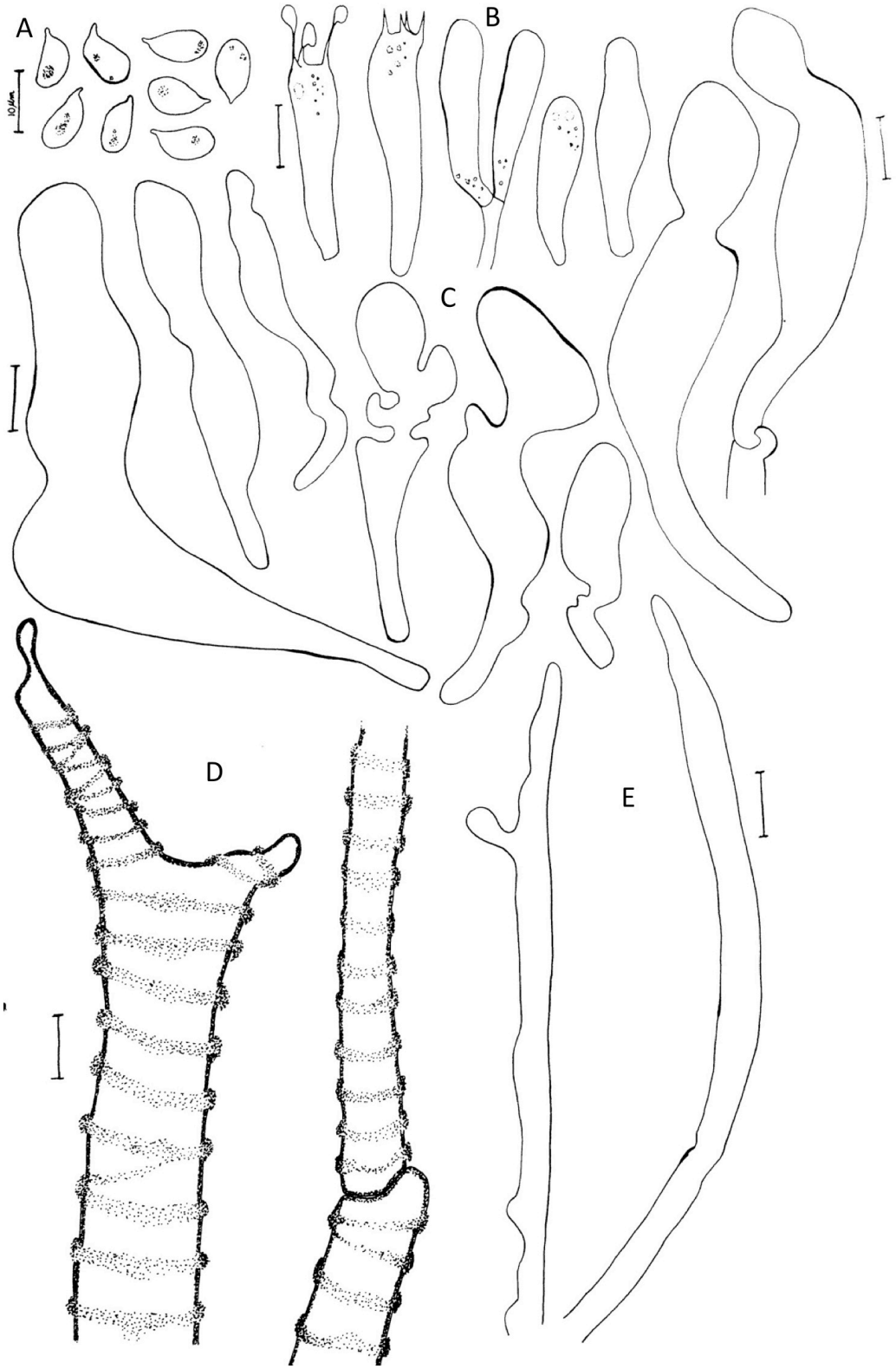


Figure 11- *Gymnopus* sp. 6

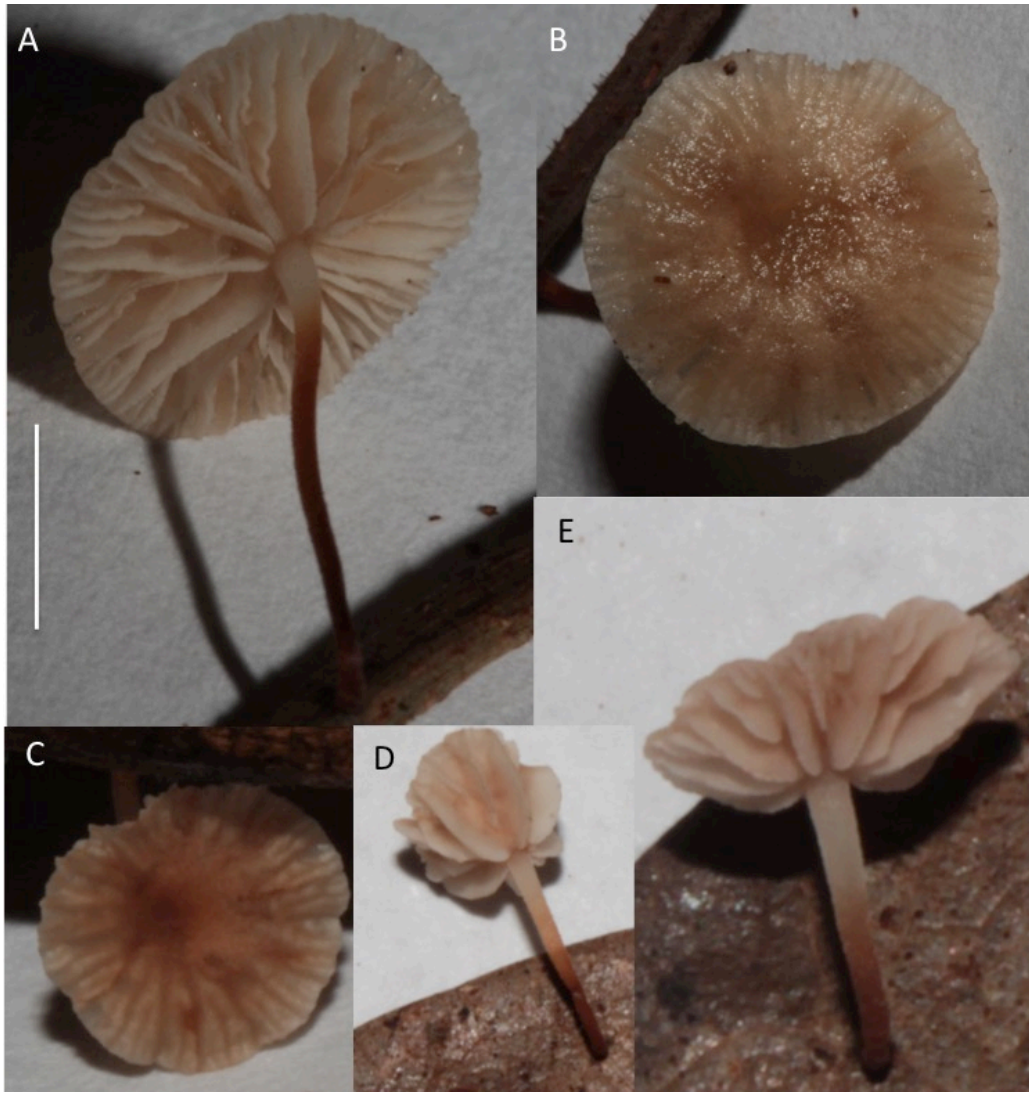


Figure 12a- *Gymnopus* sp. 4

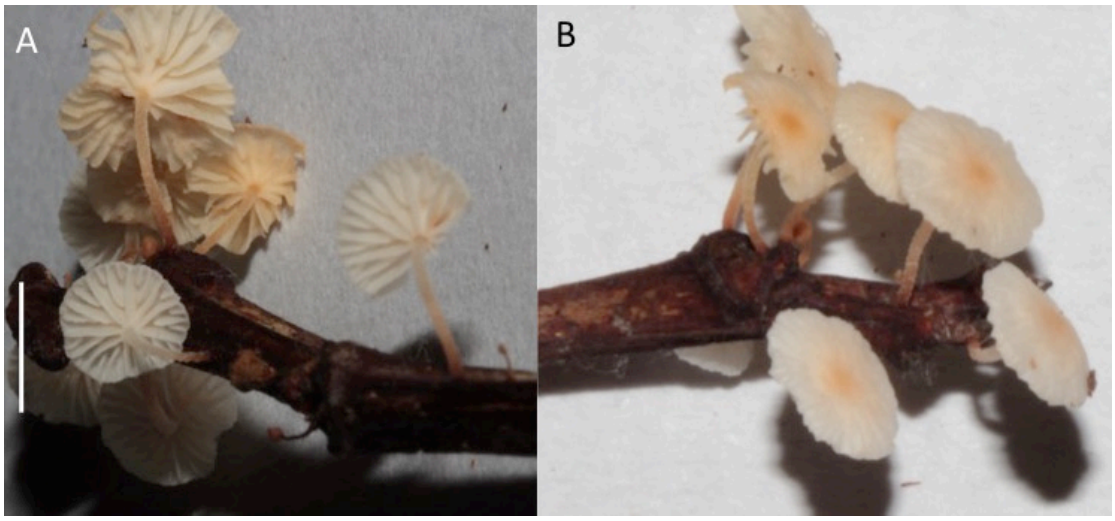


Figure 12b- *Gymnopus* sp. 4

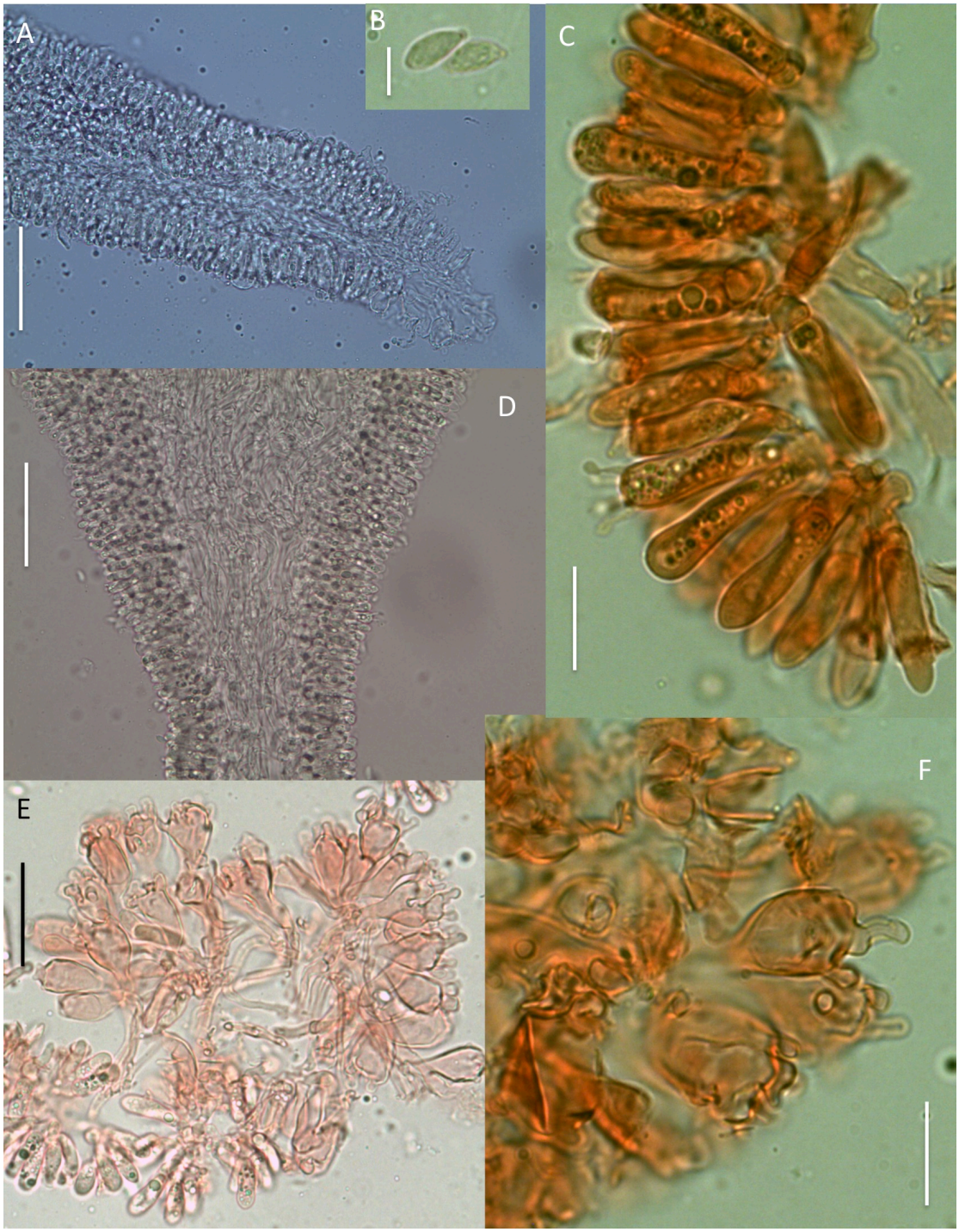


Figure 13a- *Gymnopus* sp. 14

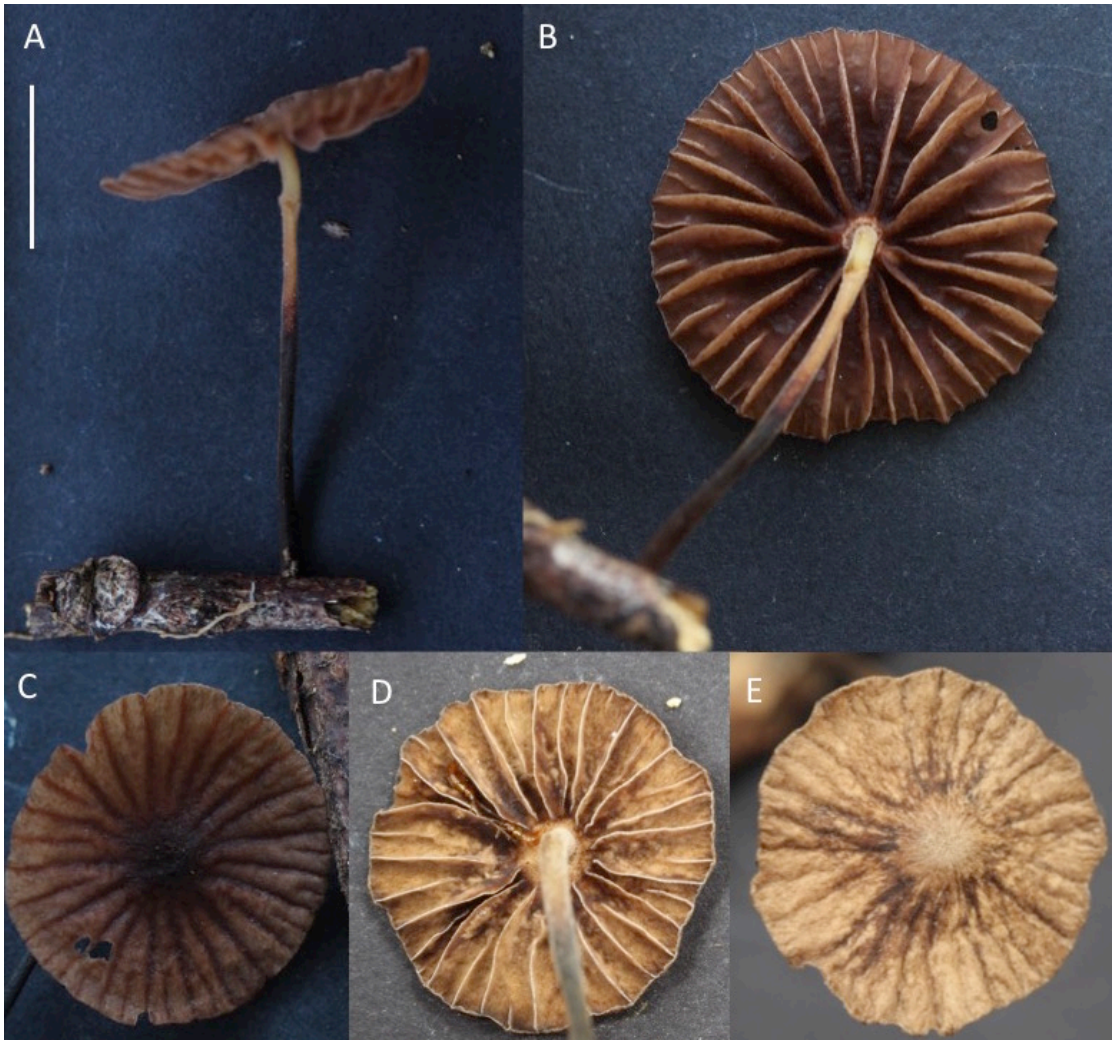


Figure 13b- *Gymnopus* sp. 14

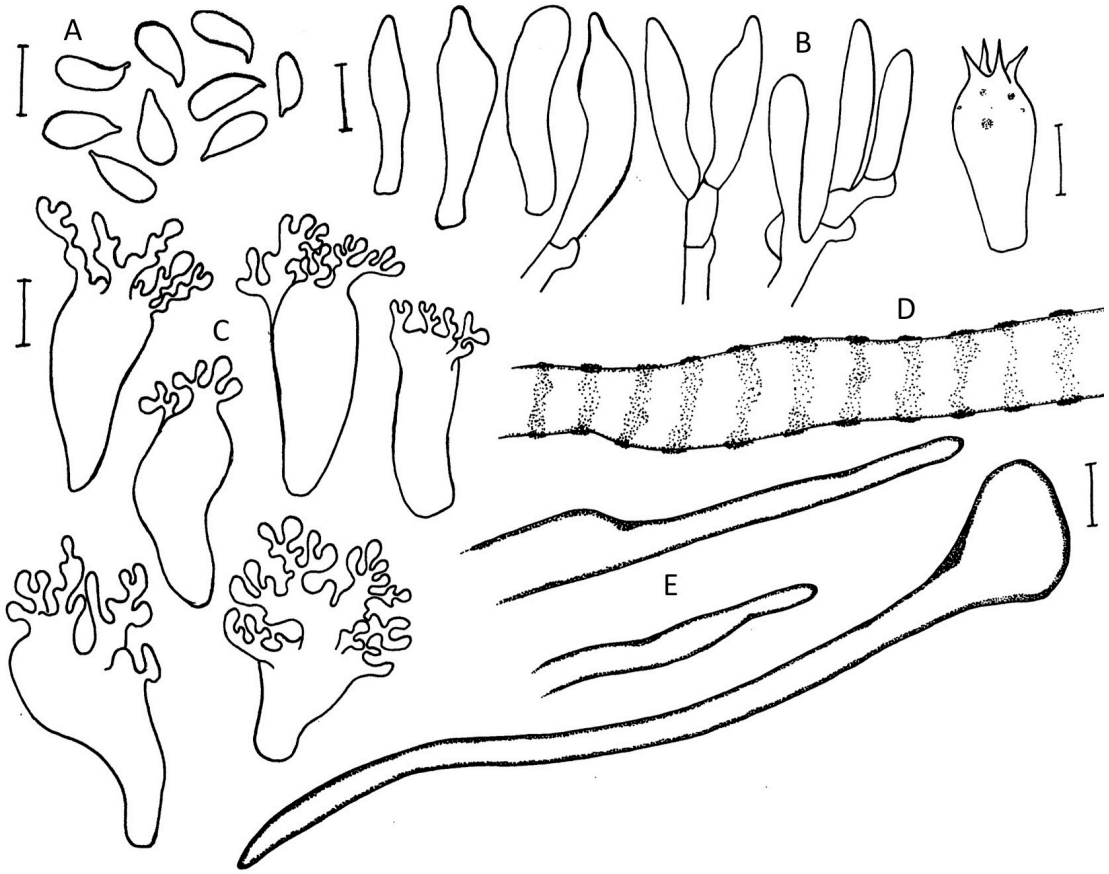


Figure 14a- *Gymnopus* sp. 17

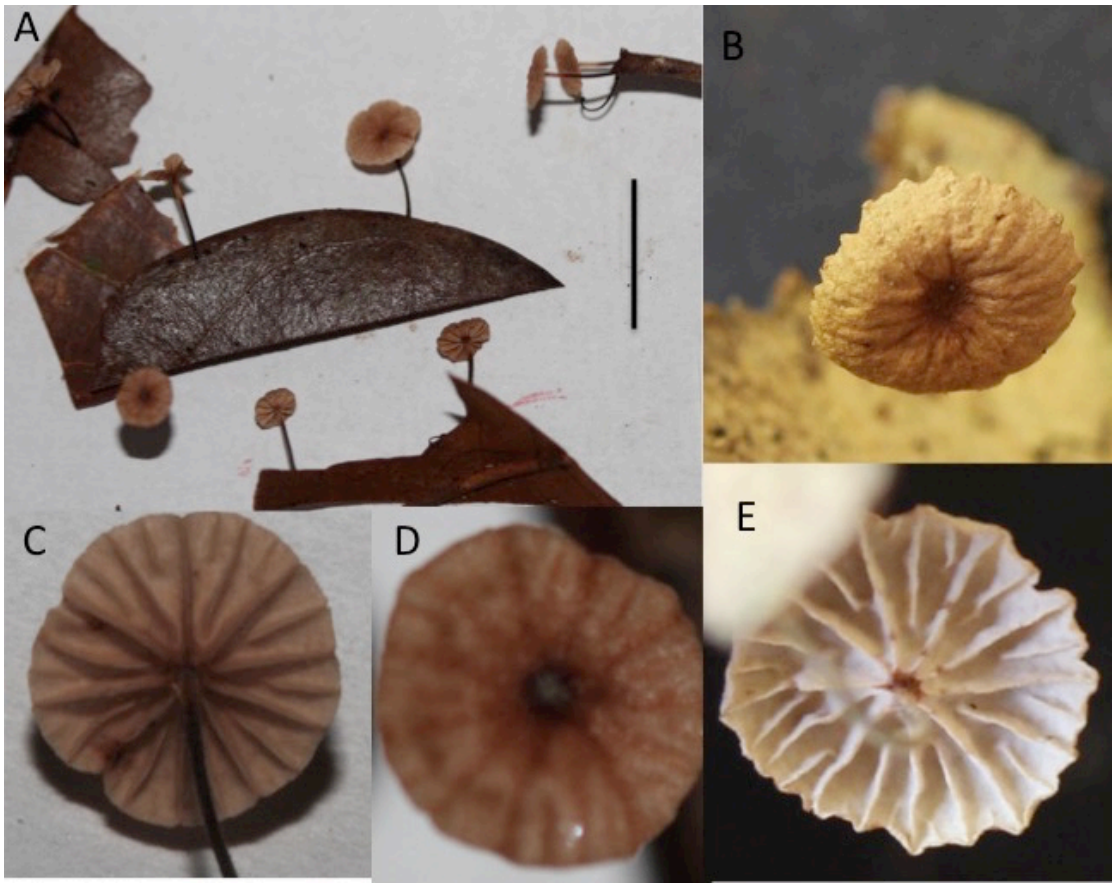


Figure 14b- *Gymnopus* sp. 17

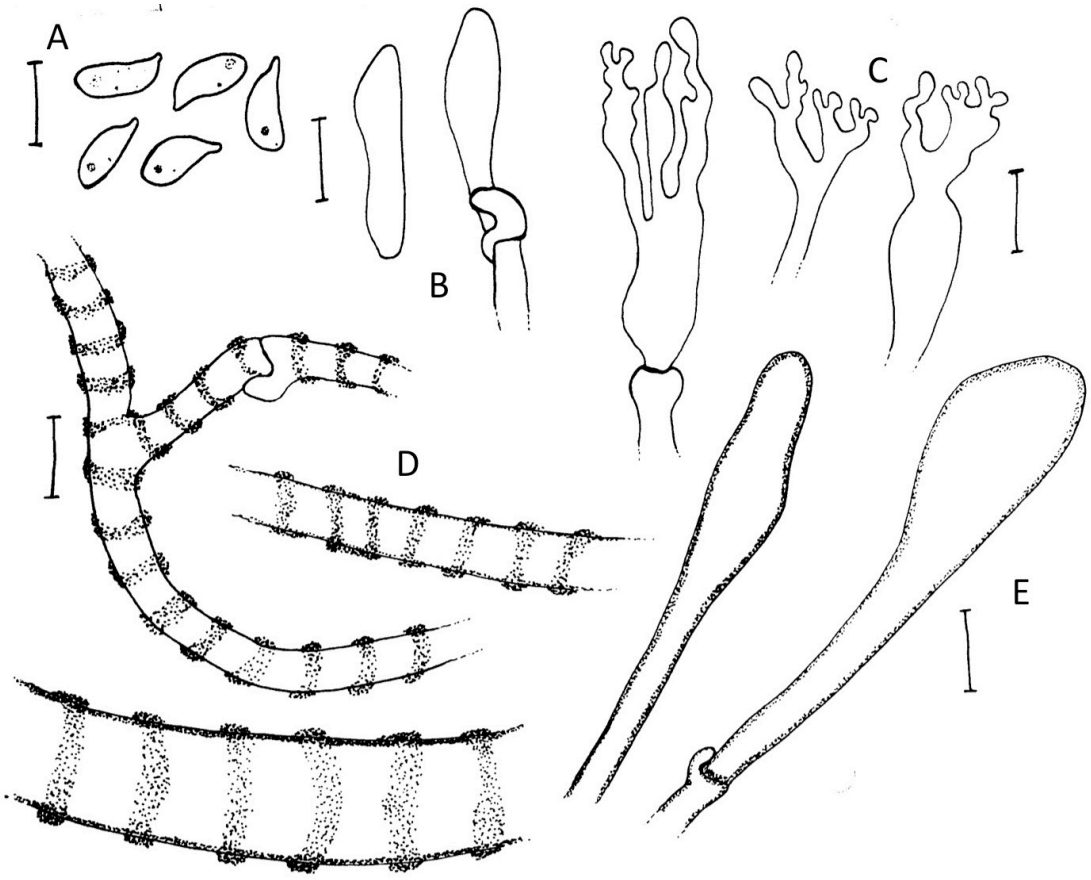


Figure 15- *Gymnopus* sp. 9

Figure 16a- *Gymnopus* sp. 20

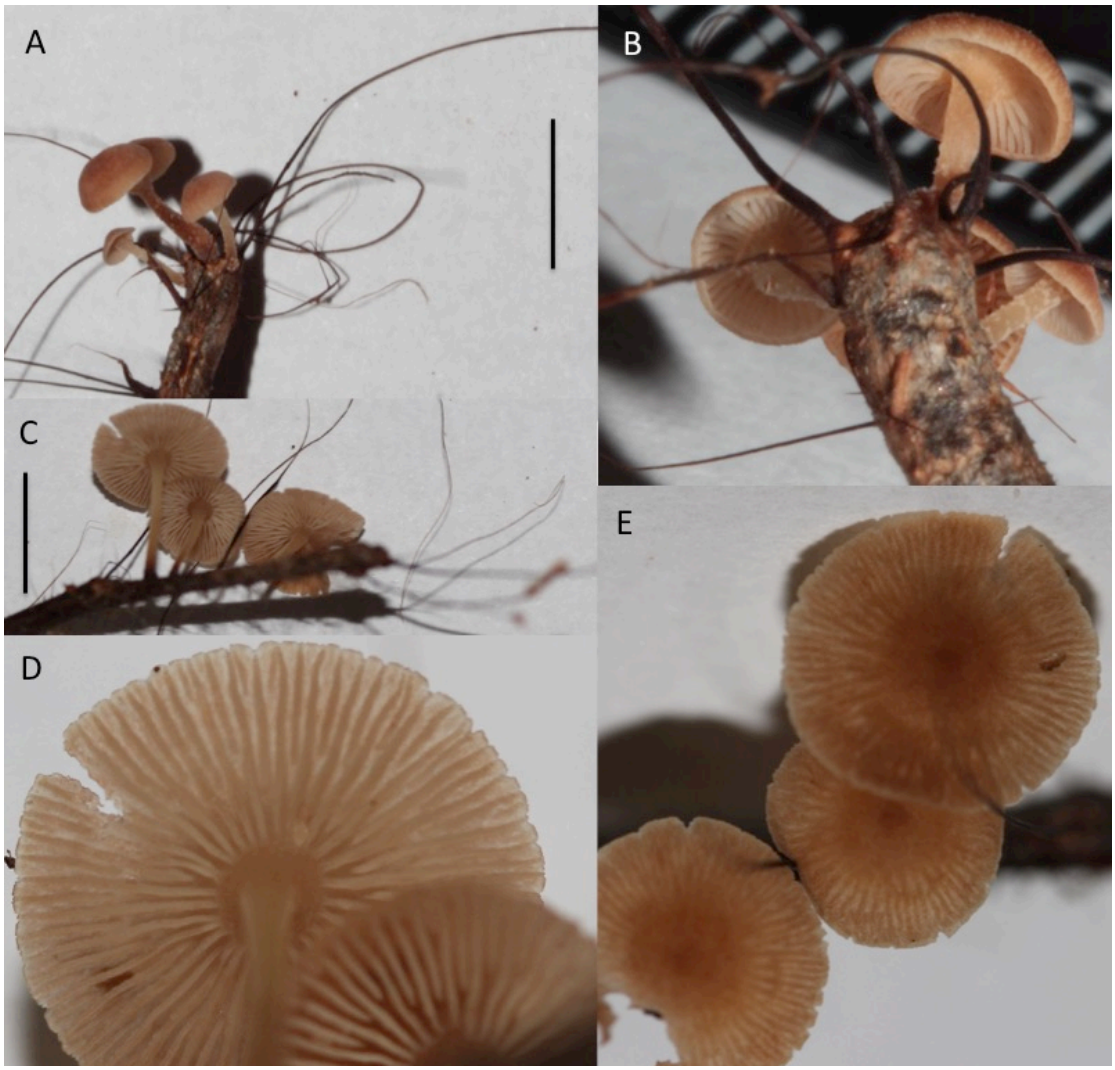


Figure 16b- *Gymnopus* sp. 20

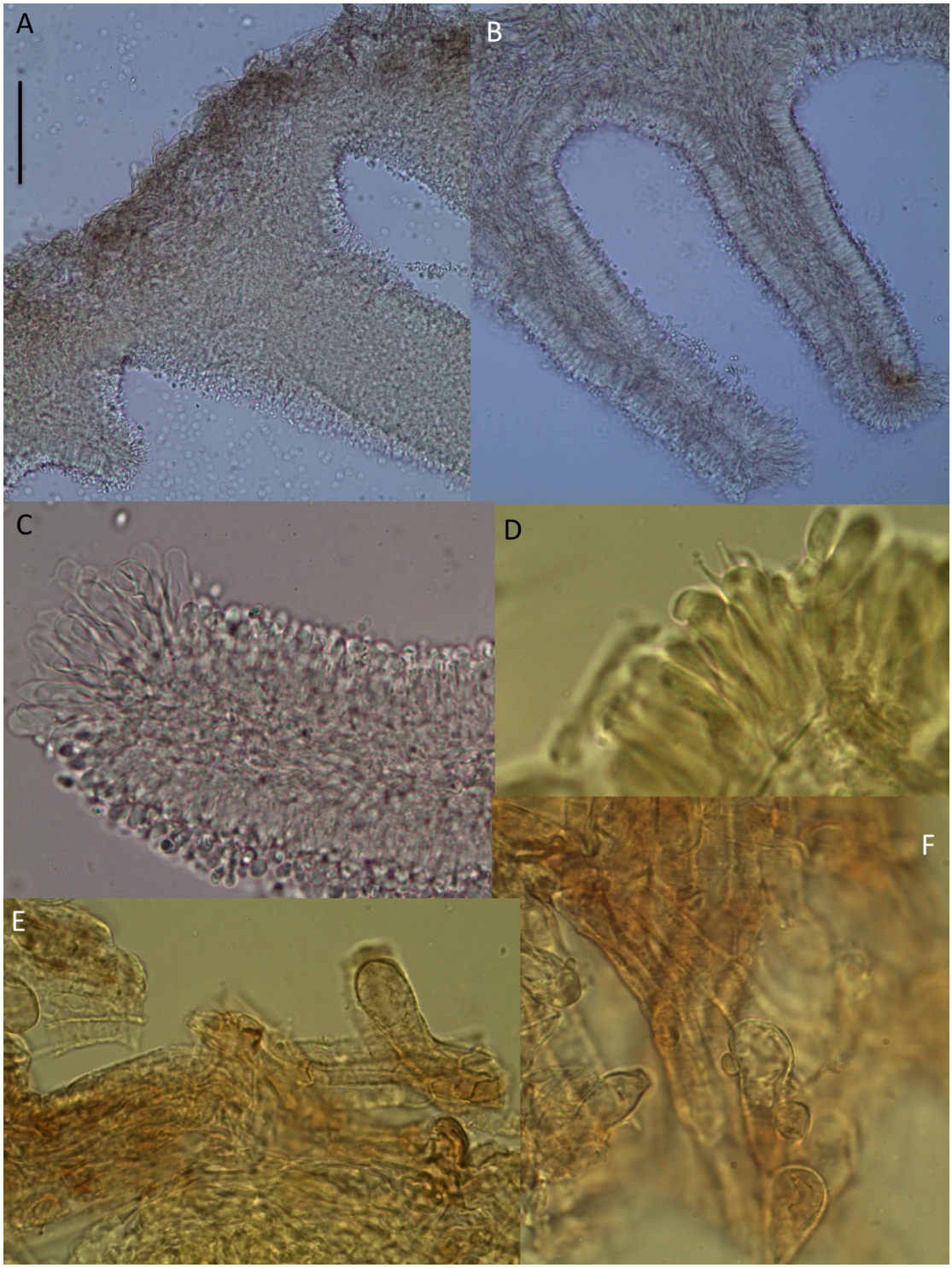


Figure 17- *Rhodocollybia* sp. 1

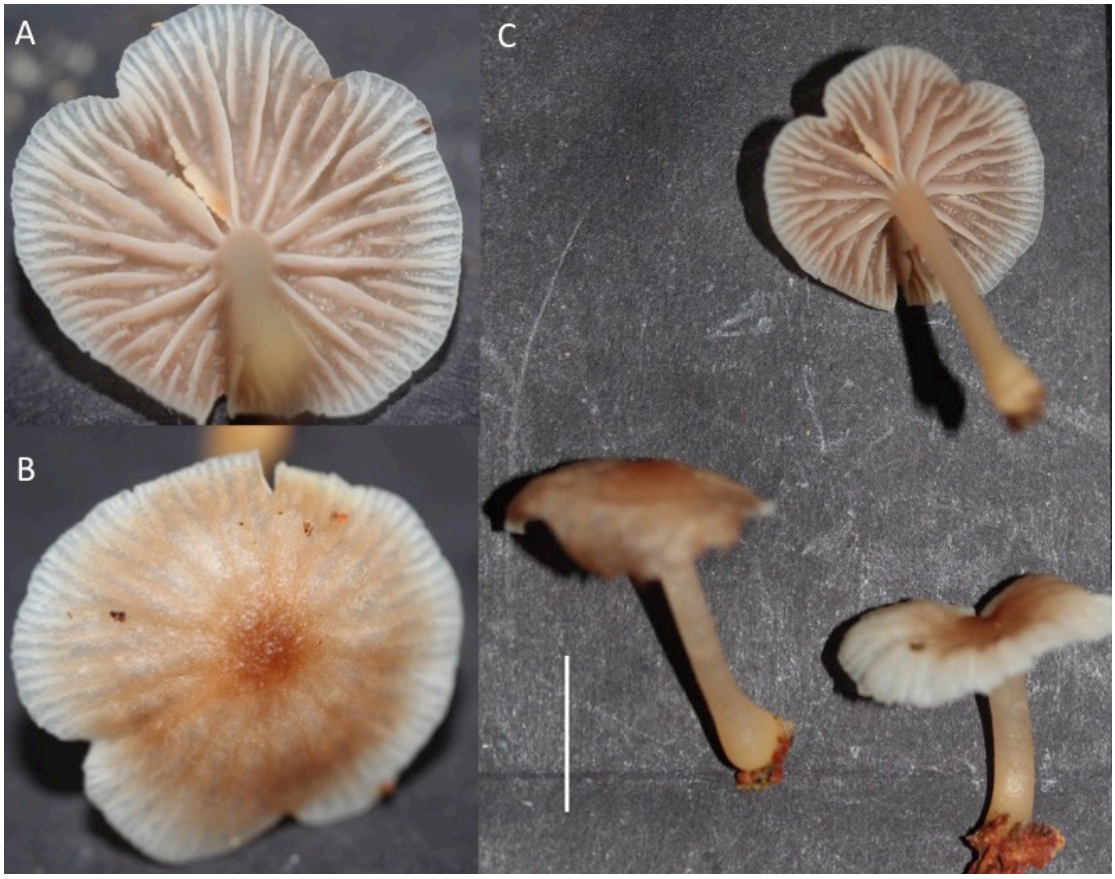


Figure 18- *Marasmiellus* sp. 1

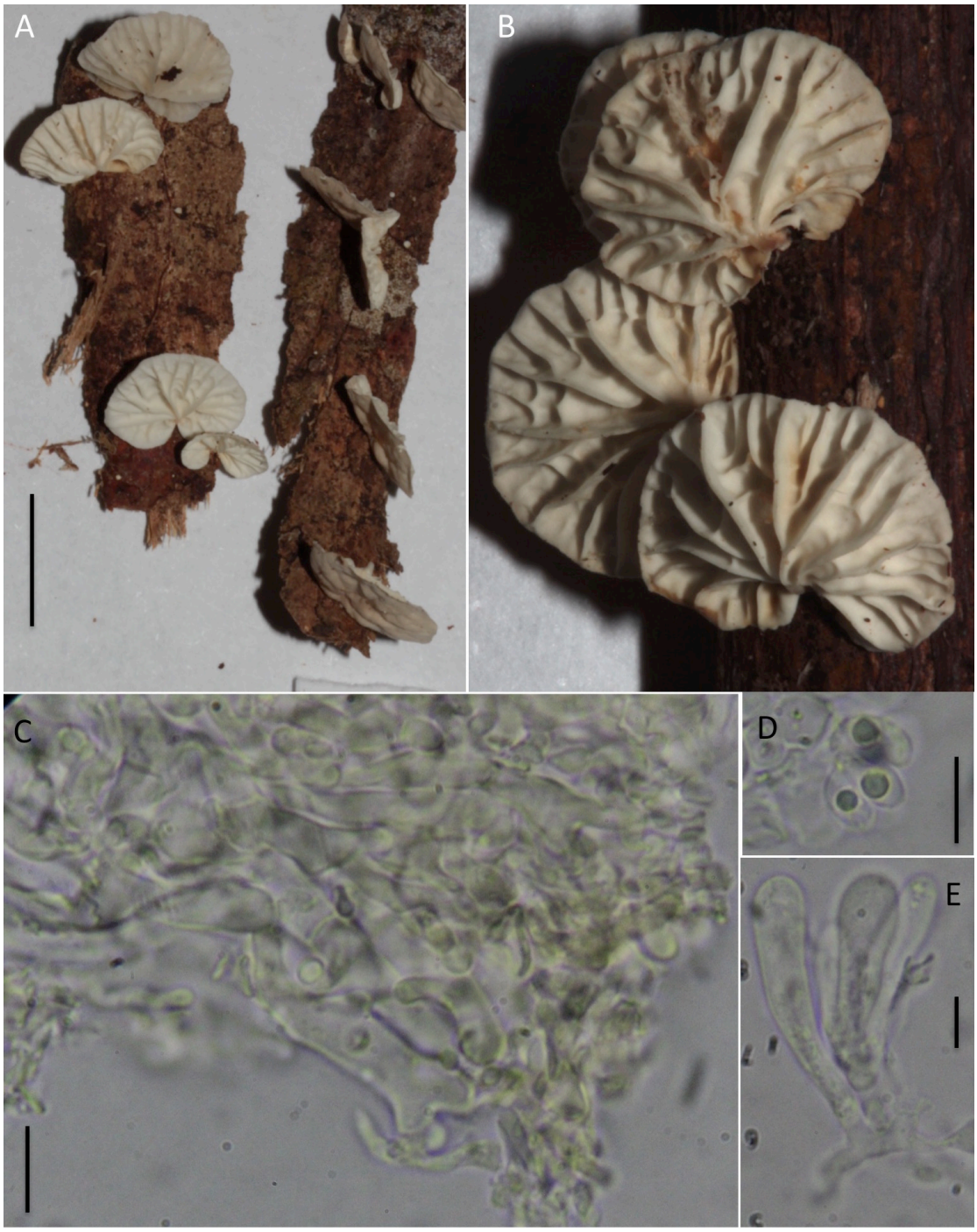


Figure 19a- *Marasmiellus* “white4”

Figure 19b- *Marasmiellus* “white4”

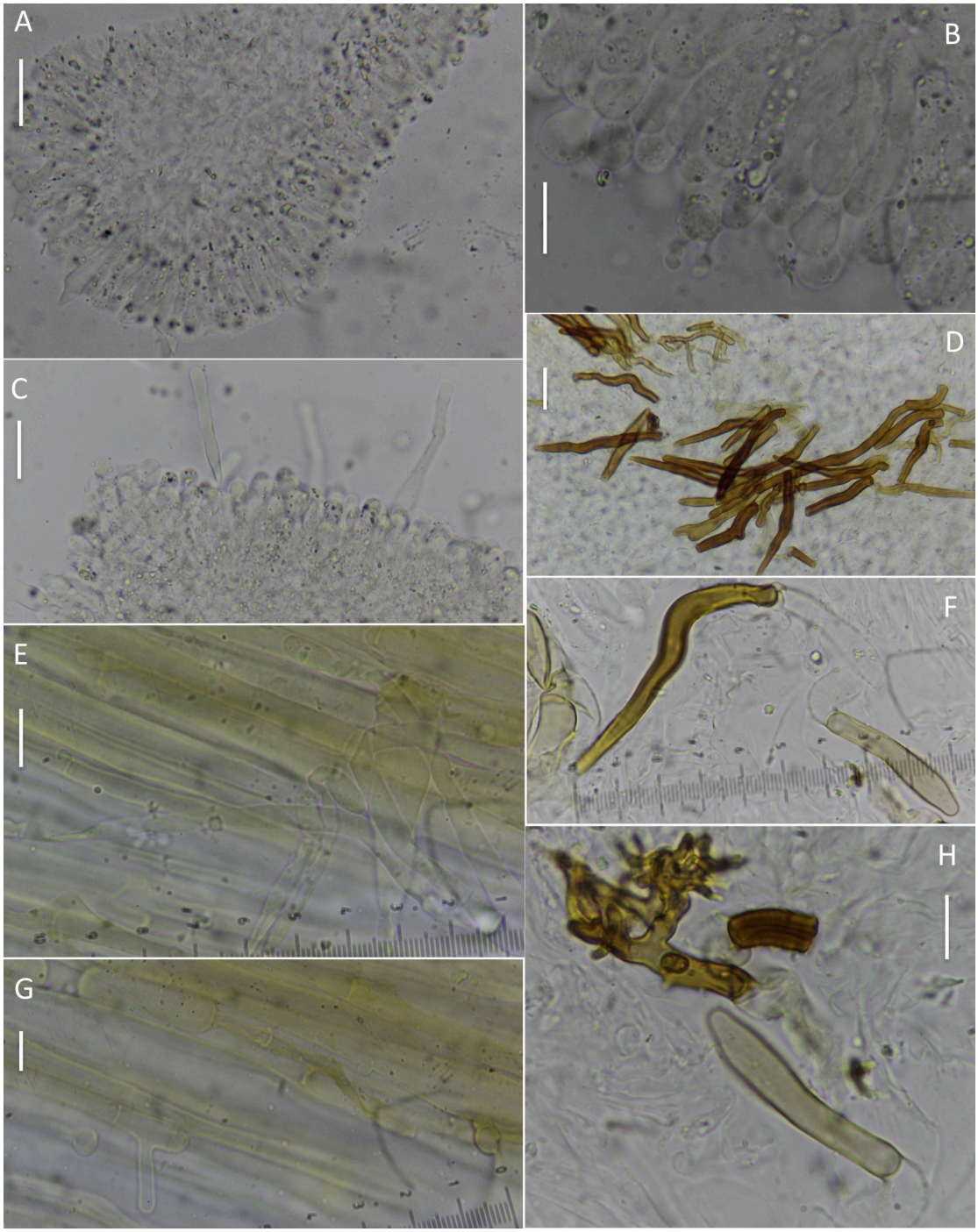


Figure 20- *Marasmiellus* “brown4”

Figure 21a- *Marasmiellus* sp. 8

Figure 21b- *Marasmiellus* sp. 8

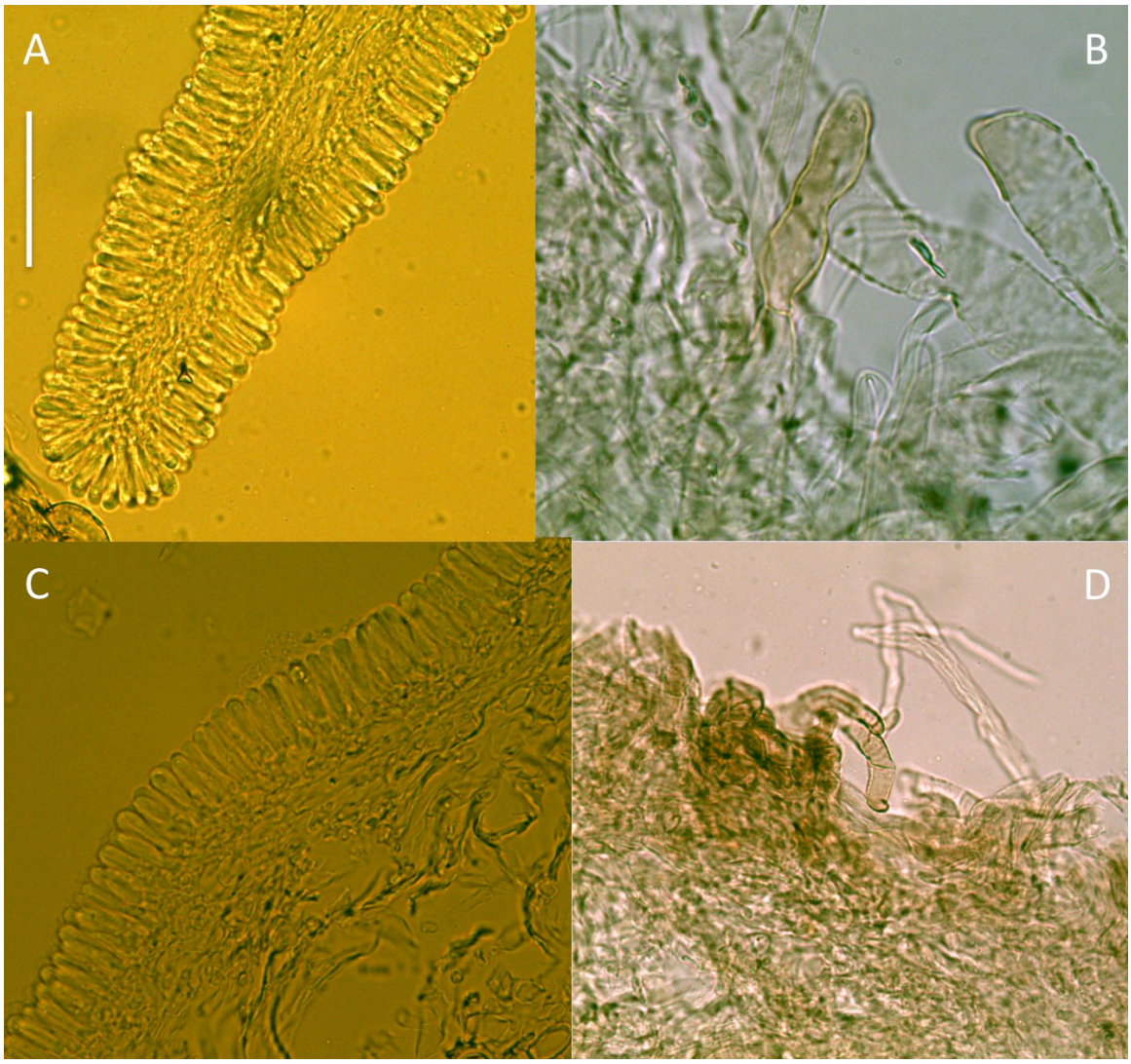


Figure 22- *Marasmiellus cubensis*

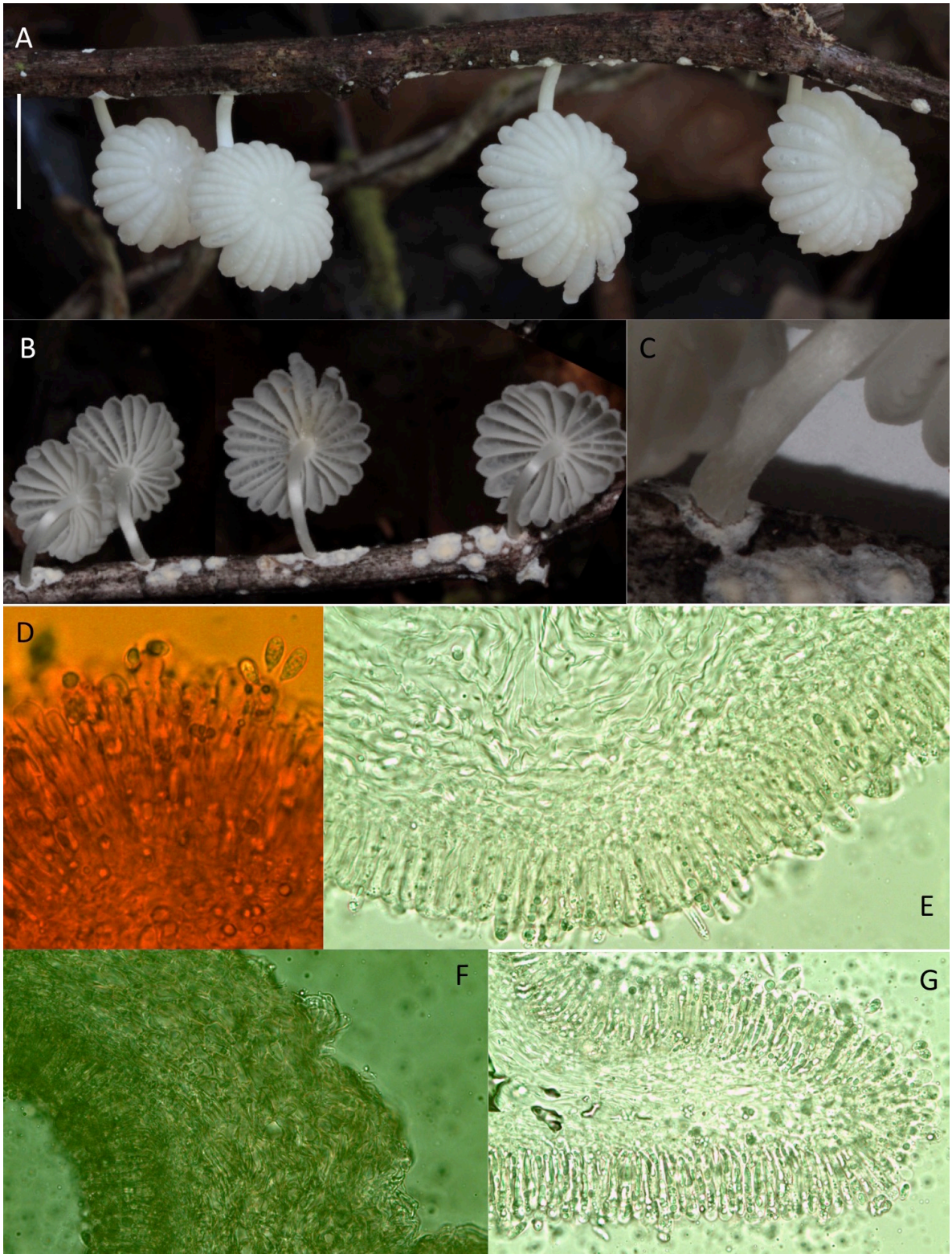


Figure 23- *Maramiellus volvatus*

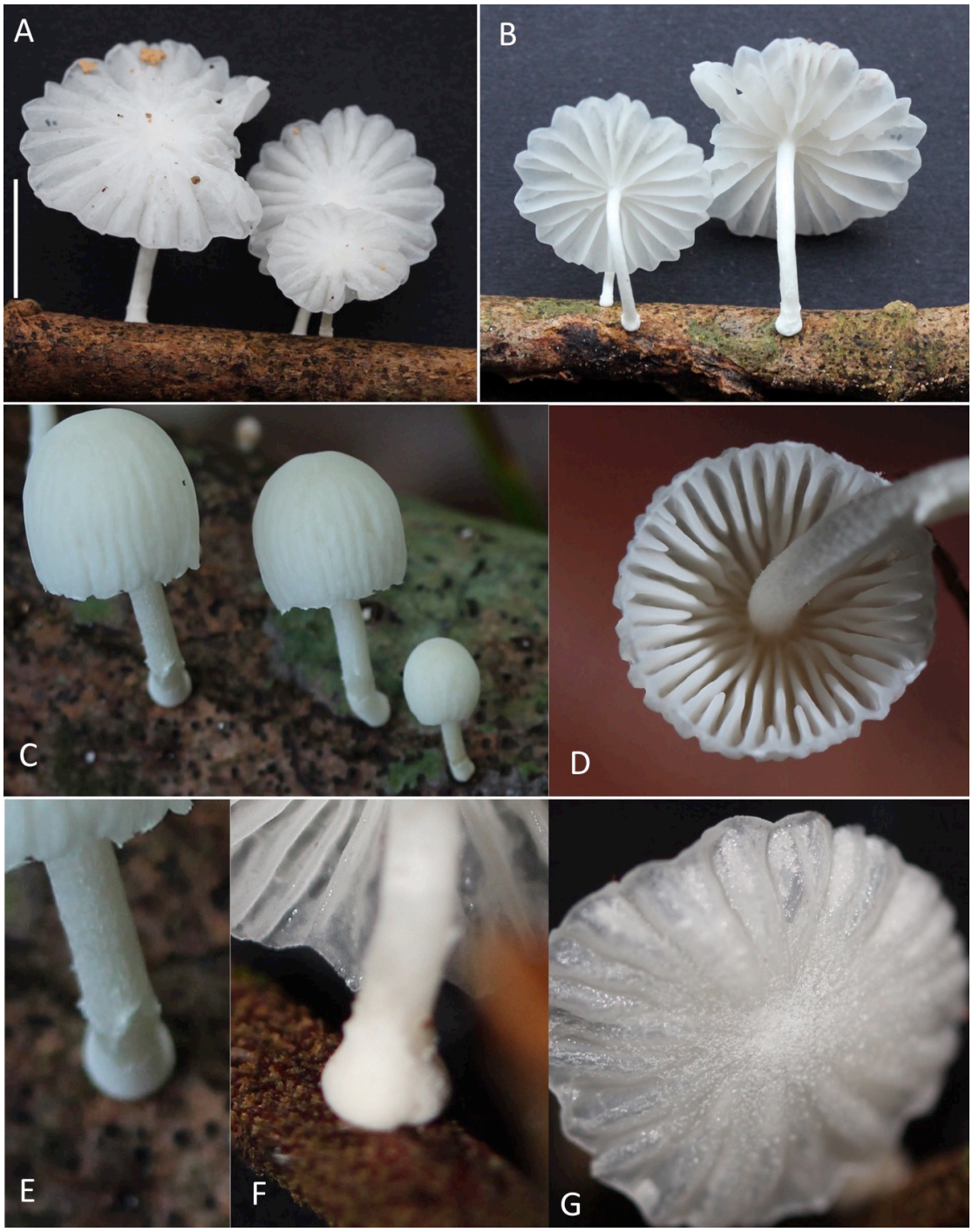
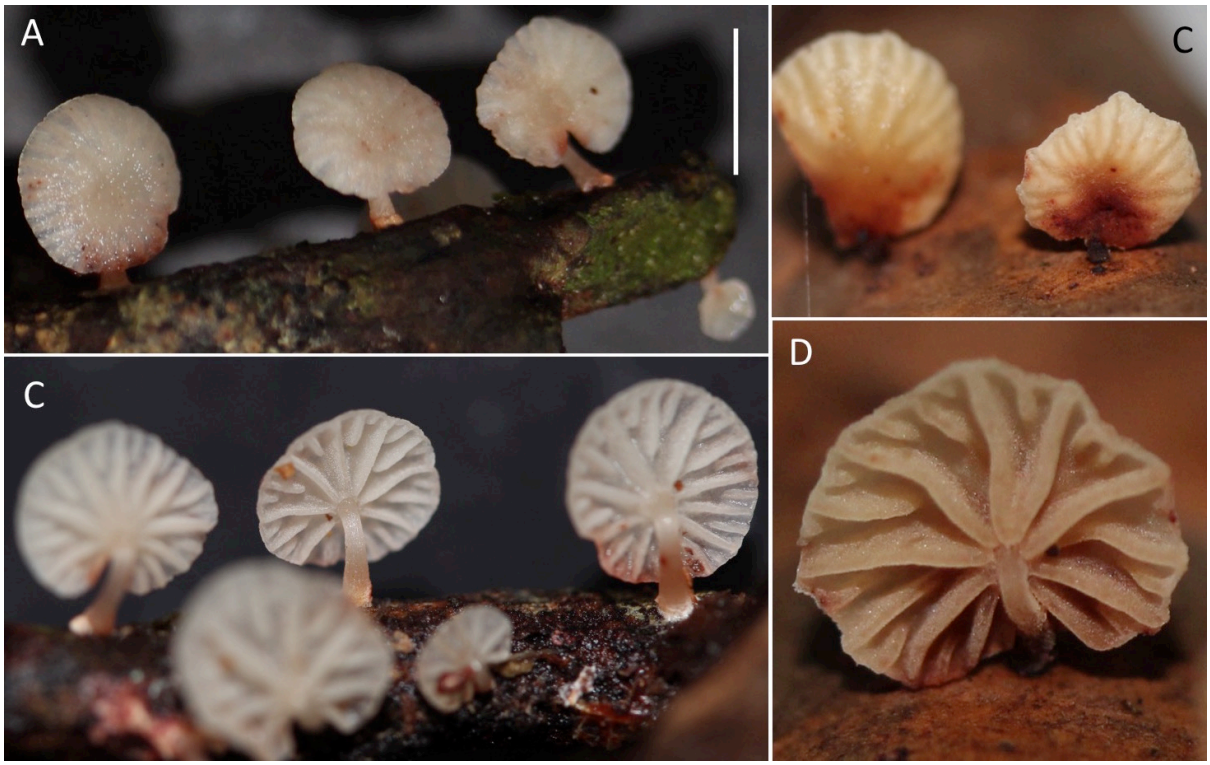


Figure 24- *Marasmiellus ramealis* var. *tucumanensis*



5- SÍNTESE

Este estudo sobre estudos ecológicos e taxonômicos de macrofungos Agaricales em área de manejo florestal permitiu que fossem testadas algumas questões e hipóteses apontadas na Introdução e nos capítulos de resultados, e possibilitou que surgissem novas questões e indicadores sobre como aprimorar estudos futuros. Além disso, como pesquisadora, pude aprofundar meus conhecimentos acerca da complexidade e dimensão dos macrofungos da ordem Agaricales, enfocando algumas de suas peculiaridades, sua importância e especialmente os desafios taxônomicos que lhes cercam.

As descrições taxonômicas e identificação dos fungos marasmioides e gymnopoides foram particularmente trabalhosas devido à grande quantidade de espécies, existência de basidiomas de dimensões reduzidas (comparados com outros grupos de cogumelos) e necessidade de distinção de caracteres microcópicos para sua identificação.

Em relação às questões ecológicas de Agaricales discutidas no Capítulo I, a hipótese de que a floresta de terra firme secundária abrigaria uma menor diversidade de macrofungos Agaricales em relação à floresta de terra firme primária não foi totalmente corroborada. Isso foi demonstrado pela análise dos macrofungos separados por substrato, o que levou à observação de que a diversidade de macrofungos no substrato "solo" foi maior na floresta primária, enquanto que para o substrato "folha" a diversidade foi, ao contrário do que se esperava, maior na floresta secundária. Além disso, foi importante considerar os efeitos sinérgicos entre as estações chuvosa e seca, uma vez que, nesta última, foi observada uma presença bem menor de basidiomas na floresta secundária.

Ainda do ponto de vista ecológico, embora mais áreas e um período maior de monitoramento pudessem ampliar o entendimento do comportamento e distribuição de macrofungos, nossa identificação de 287 táxons em escala local e num período de dois anos já explicita de maneira pronunciada o quão rica é a diversidade da área de manejo estudada. Neste sentido, o atual trabalho tem o potencial de nortear futuros estudos micológicos na Amazônia, pois sugere a importânicia de se considerar especificamente os substratos e a sazonalidade pluviométrica de um modo mais criterioso e menos intuitivo.

Quanto aos estudos taxonômicos, a incorporação de dados moleculares como o ITS foi importante para a identificação das espécies. O ITS, embora apresente algumas limitações, foi recentemente preconizado como a sequência de *DNA-barcode* para todos os grupos de fungos (Schoch et al. 2012), motivo pelo qual o utilizamos para delimitar as espécies em conjunto com as análises morfológicas. No entanto, sua utilização ocorreu com cautela, uma vez que o ITS é extrematente variável e apenas com seu adequado alinhamento é possível obter análises

mais acuradas. Para inferências evolutivas, que não foi o escopo do trabalho, seria necessária a pesquisa de outras regiões gênicas.

Assim, com dados moleculares e morfológicos obtivemos os resultados do Capítulo 2, no qual seis espécies novas de *Tetrapyrgos* foram descritas. No Brasil, há somente o registro de *T. nigripes*, sendo recentemente publicada *T. longicystidiata* (Honan et al. 2015) com ocorrência para a Mata Atlântica e agora também para a Amazônia. No entanto, não é surpresa encontrar espécies novas de *Tetrapyrgos* na Amazônia, pois Singer (1973) já descrevia, mesmo que num trabalho inconcluso, pelo menos cinco formas distintas de *T. nigripes* (*Marasmiellus nigripes*).

No tocante a *Marasmius*, já era esperado que fosse majoritário nas coletas, uma vez que a diversidade deste é maior nos trópicos, sendo um dos gêneros mais numerosos depositados no herbário INPA. Para tal gênero, três capítulos foram destinados:

Captítulo 3- Descrição de espécie nova relacionada à *Marasmius horridulus*. Agora, a subseção Horriduli, peculiar dentro da seção *Marasmius*, conta com mais uma espécie, *M. calvocystidiatus*, que apresenta características muito similares a *Marasmius horridulus,* mas possui píleo liso, com cistídios clavados e sem pontas. Devido às mudanças infragenéricas na seção *Marasmius,* a inclusão desta espécie precisa ser avaliada com mais dados de sequências de espécies dessa seção para validar a subseção Horriduli (Singer 1986). A presença de píleo hirsuto em *Marasmius horridulus* pode ter gerado designações equivocadas de *Crinipellis* no Herbário INPA, o que torna pioneira nossa coleta de *M. horridulus* após sua descrição por Singer (1986).

Capítulo 4- Apresentou *Marasmius* com rizomorfos eretos formando estruturas racemosas. A análise de ITS mostrou que todos os ramos das análises filogenéticas preliminares apresentaram-se bastante longos. Em geral, o tamanho das sequências de ITS de *Marasmius* com rizomorfos deste tipo é mais longa, quando comparadas com outras sequências de ITS de *Marasmius*. A presença de rizomorfo não é um caracter distintivo, porém estas espécies apresentaram cistídios bastante diferenciados compostos por células espessadas, pigmentadas e com transições ou variações entre cistídios do tipo *Siccus* e *Rotalis*. Por estas características, tal grupo seria bastante promissor na composição de estudos evolutivos de *Marasmius*, uma vez que atualmente vêm sendo reformuladas sua delimitação de gênero e suas relações infragenéricas.

Capítulo 5- Neste último capítulo destinado a *Marasmius,* foram descritas as espécies remanescentes, até então não analisadas. Assim, trinta e sete taxa foram identificados, dos quais cerca de 57% até o nível de espécie. Nos 43% restantes enfrentamos dificuldades para completa identificação porque em parte dos materiais não foi possível visualizar as estruturas

microscópicas. Além disso, em vista da dimensão deste grupo e a existência de muitos padrões morfológicos sobrepostos, nossa familiaridade com o material foi insuficiente para delimitações mais refinadas. De qualquer forma, ressaltamos que para todos os materiais foram produzidos dados de ITS, descrição macro e microscópica e pranchas com imagens dos fungos estudados, dando base para posteriores trabalhos.

O Capítulo 6 destinou-se aos fungos *Gymnopus*, *Rhodocollybia* e *Marasmiellus*, atualmente aceitos como pertencentes a Omphalotaceae. Contudo, esses gêneros não formam grupos monofiléticos e estudos filogenéticos têm mudado amplamente sua classificação. *Gymnopus* e *Marasmiellus* são gêneros bastante especiosos, diversos principalmente nos trópicos e com poucas características marcantes que possam delimitar o grupo. Mas como nosso objetivo com a tese se restringiu a um trabalho majoritariamente ecológico, a morfotipação de *Gymnopus* e *Marasmiellus* até o nível de gênero que alcançamos foi suficiente. Consequentemente e, de acordo com nossa previsão, o aprofundamento do estudo taxonômico para estes dois grupos será alvo de um projeto de pós-doutoramento.

Importante salientar que no Capítulo 1 desta tese indicamos que seria interessante se os trabalhos ecológicos de macrofungos fossem aliados a outros trabalhos ecológicos (não necessariamente sobre fungos) de longa duração, para que desse modo sejam melhor compreendidos aspectos ecológicos dos fungos como fenologia, dispersão, entre outros.

Por outro lado, como a literatura é ampla e notoriamente fragmentada, destacamos a importância da criação de guias taxonômicos contendo atualizações da nomenclatura em vigor da ordem Agaricales que ocorrem na Amazônia com descrições, imagens e ilustrações de macrofungos, o que otimizaria a identificação de espécies.

Em suma, por meio desta tese, os dados moleculares do ITS (incluídos no GenBank), as imagens de fungos cuidadosamente produzidas e os respectivos materiais depositados no Herbário INPA tornaram mais acessíveis espécimes restritas a áreas de pouco acesso a muitos especialistas de macrofungos Agaricales em todo mundo.

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ANEXOS
ANEXO I: FTA card extraction method

by Simona Margaritescu By Simona Margaritescu- Royal Ontario Museum – LMS Protocol for Mycology

Sample DNA from fresh mushrooms following the instruction in the video https://www.youtube.com/watch?v=Gir56iYspTE .

When DNA is needed, obtain samples by punching the FTA card with a Harris Uni-Core tip (Figure1) and placing each sample in the well of a 96-well PCR plate.

Add 25 µL of Extraction solution (Sigma Extract-N-Amp) to each well and then incubate the plate in a thermal cycler set at 95 C for 10 min.

After the incubation, an equal volume (25 µL) of Dilution solution is added to the reaction to terminate the extraction reaction.

From this, a 1:10 to 1:50 dilution in water is used for PCR (should be determined empirically, but 1:10 generally works well). The proprietary reagents used in the extraction kit inhibit standard PCR reactions unless they are diluted beforehand.

Materials used

- PCR 96-well plate
- Strip caps
- Whatman™ WB100038 Harris Uni-Core™ Micro-Punch with 3mm I.D. Tip for FTA™ Card Samples
- Whatman™ WB100020 FTA™ Card Cutting Mat for Harris Micro-Punch & Uni-Core™ Punches, 5.9 x 7.9-Inch
- Sigma Extract-N-Amp™ Plant PCR Kit (see PDF for more info)
- Whatman™ WB120065 FTA™ PlantSaver Card (100 cards/pack)

Resource:

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

Figure 1 – Harri Micro-Punch 2.00 mm and instructions

Harris MICRO-PUNCH® 2.00 mm

"cuts, retrieves, and stores cored samples in one operation"

The Harris Micro-Punch® is designed to cut, retrieve, store and preferentially eject cored samples from source materials such as paper, gels, cloth, leaves, paint chips, films, etc. The tips and plungers are made from high grade 440c stainless steel. Tips are heat treated to Rockwell hardness Rc 58 and then individually sharpened to a razor edge for a long cutting life. Tips are available in diameters of 0.50, 1.00, 1.20, 2.00 and 3.00 mm. The barrel and knob-screw are Swiss-style machined from high grade, 6061 lightweight aluminum and anodized to form a protective, chemically resistant, long lasting surface for easy handling. Each Harris Micro-Punch® is sold with a protective plastic tip cover, instruction sheet and storage container. A 6 x 8", 1.5 mm thick, inert, self-healing cutting mat with dual cutting surfaces is sold separately and is required for most applications. Replacement tips and mats are available on request.

Sample Extraction - **(DO NOT DEPRESS PLUNGER WHEN CORING SAMPLE)**. Position source material (i.e. blood cards, paint chips, paper, leaves, gels, etc.) on self-healing cutting mat. Remove protective tip cover cap from Harris Micro-Punch™ with gentle twisting. Firmly grip Harris Micro-Punch™ as shown, or in any other comfortable position. The tip is positioned at a right angle above the target area **(DO NOT DEPRESS PLUNGER)**. Use the free hand to hold the mat and material steady. The tip is pushed downward into the source material with a semi-circular rotation until the tip passes through the source material and makes contact with the cutting mat. Lift the Harris Micro-Punch® away from the source material with the cored sample stored in the cutting tip.

Sample Storage and Ejection - After coring, the sample is temporarily stored in the hollow cutting tip ready for preferred ejection. The Harris Micro-Punch® can be rested on its side without loss of sample from the hollow tip. To eject sample, position tip over target area and depress plunger as shown.

Suggested Cleaning - Clean tip between each sample extraction by coring blank filter paper; rinsing with ethanol or spraying with compressed air to remove dried artefacts. Rinse mat with ethanol after each sample extraction.

FOR LABORATORY USE ONLY.

ANEXO II: DNA Extraction protocol in plates using glass fiber AcroPrep-PALL plates

By Simona Margaritescu- Royal Ontario Museum – LMS Protocol for Mycology

Before you begin

A: Prepare **Binding Mix (BM).**

Stock solutions	25 ml for 2 plates	
Binding Buffer (see Table I)	12.5ml	25ml
EtOH 96%	12.5ml	25ml
Total volume	25ml	50 ml

BM can be stored at room temperature for one week.

B: Prepare **Protein Wash Buffer (PWB)**.

Stock solutions	50 ml for two plates	
Binding Buffer (see Table I)	13ml	26ml
EtOH 96%	35ml	70ml
ddH2O	2ml	4ml
Total volume	50ml	100ml

PWB is stable at room temperature for approximately one week; discard if any crystallization occurs or better still: make it fresh every time!

Protocol

1. Add a small amount of sample to each well of a 96-well plate; cover each column with caps as you add samples.

2. For one plate, mix 5ml of Vertebrate Lysis buffer and 0.5ml Proteinase K, 20 mg/ml in a sterile container. Add 50ul of resulting Lysis Mix to each well of the 96-well Eppendorf plate.

3. Incubate at 56°C for a minimum of 6 hours or overnight to allow digestion.

4. Centrifuge about 30 seconds (program 8) to remove any condensate from the cap strips or spin by hand in the home made contraption or salad-spinner.

5. Add 100ul of Binding Mix to each sample using multichannel pipette and mix content by pipetting slowly up and down. Cover plate with cap strips and centrifuge for about 20 seconds (program 8) to sediment the lysate. Remove cap strips/cover and transfer the lysate (about 150ul) from the wells of microplate into the wells of the PALL Plate placed on top of a square-well block (or set-up the vacuum system) using multichannel pipette. Seal the plate with adhesive cover.

6. Centrifuge at 1500 xg for 10 minutes to bind DNA to the glass-fiber (GF) membrane. Alternatively, apply vacuum until all liquid went through.

7. **First wash step**: add 250 ul of Protein Wash Buffer (PWB) to each well of the GF plate (use 300ul Pipette and 300 ul tips). Seal with a new adhesive cover and centrifuge at 1500 xg for 5 min or apply vacuum again until all liquid went through.

8. **Second wash step**: add 300 ul of Wash Buffer (WB) to each well of the GF plate. Seal with a new cover and centrifuge at 1500 xg for 10 min or vacuum through.

9. **Third wash step**: add 300 ul of Wash Buffer (WB) to each well of the GF plate. Seal with a new cover and centrifuge at 1500 xg for 10 min or vacuum through.

10. If you see that WB did not go through the membrane in all samples, open the cover, seal the plate again and repeat the centrifuge step at 1500 xg for 10 minutes or vacuum again.

11. Remove the cover. Place the GF plate on the lid of a tip box. Incubate at 56°C for 30 min to evaporate residual ethanol [cover with a KimWipe - optional].

12. Take a clean PCR rack, position a PALL collar on the collection plate and place plate and collar on top of the rack [collar will help to take the Eppendorf plate with eluted DNA out of the rack]. Place PALL plate with DNA bonded to the membrane on top of the regular Eppendorf plate [by now the Eppendorf plate-collar-square block trio has been put together]. Dispense 50ul of ddH2O (**PREWARMED TO 56°C**) directly onto the membrane in each well of GF plate and incubate at room temperature for several minutes. Seal plate. *As an extra caution, use tape to hold together the glass fiber PALL plate, Eppendorf plate and rack*.

13. Centrifuge at 1500xg for 10 minutes to collect the DNA eluent. Remove the GF plate and discard it.

14. Cover DNA plate with cap-strips. Store at -20°C.

Table 1. Working solutions for DNA extraction.

Vertebrate Lysis Buffer (VLB)			
Stock	**Volume/amount**	**Volume/amount**	**Final concentration**
1M NaCl	10 ml	5 ml	100 mM NaCl
1M Tris-HCl, **pH 8**	5 ml	2.5 ml	50 mM Tris-HCl, pH 8
0.5M EDTA, pH 8	2 ml	1 ml	10mM EDTA, pH8
SDS	0.5 g	0.25 g	0.5% SDS
ddH2O	83 ml	41.5 ml	
Note: mix on magnetic stirrer with heater; buffer can be stored			
Total volume	100 ml [20 plates]	50 ml	
Binding Buffer (BB) (need 52 ml for 2 plates, make 75 ml)			
Stock	**Volume/amount**	**Volume/amount**	**Final concentration**
GuSCN	35.46g	17.23g	6M GuSCN
0.5M EDTA, pH 8	2ml	1 ml	20 mM EDTA, pH 8
01M Tris-HCl, **pH6.4**	5ml	2.5 ml	10mM Tris-HCl, **pH6.4**
Triton X-100	2ml	1 ml	4% Triton X-100
ddH2O	As needed	As needed	
Total volume	50 ml	25 ml	

Note: mix on magnetic stirrer with heater; buffer can be stored, but crystals will most likely form. If they do, pre-warm at 56C to dissolve before use.			
Wash Buffer (WB) For 475 ml (aliqot in 50 ml tubes, keep at -20°C)			
Stock	**Volume/amount**	**Final concentration**	
EtOH 96%	300 ml	60% EtOH	
1M NaCl	23.75 ml	50mM NaCl	
1M Tris-HCl, **pH7.4**	4.75 ml	10mMTris-HCl,**pH 7.4**	
0.5M EDTA, pH 8	0.475 ml	0.5mM EDTA, pH 8	
ddH2O	146.025 ml		
Note: mix well, store at -20C			

Note: Square blocks can be washed with ELIMINase, autoclaved and used again.

Anexo III: PCR purification using the pipette tip method

By Simona Margaritescu- Royal Ontario Museum – LMS Protocol for Mycology

Make an agarose gel (1%-1.2% agarose) and use the 24-well, thick combs (1.5 mm thick). For example, make a 100 ml gel and add 10 µl SYBR Safe.

Load the whole volume of the PCR product on the gel and run it at 90-100 V for at least 30 minutes (the bigger the fragment the longer the run).

Cut off the end of a filtered pipette tip (i.e. Progene 200 µl filter tips, cat# 24-TF200-RS) with a clean razor blade. Make the cut at the second notch from the tip. Place the cut tip into an Eppendorf tube and label the tube.

Take your gel into the darkroom. Cut out the band and carefully place it into the filter tip, on top of the filter. Try to cut the band as accurate as possible, thinner bands will give you less liquid therefore less dilution of you PCR product (aim for a final volume of <50 µl). Rinse (use ddH_2O) and wipe the blade after each band.

Centrifuge for 10 minutes at full speed (regular bench centrifuge, 13,000 rpm).

When done, pull out the tip with a pair of tweezers. Discard the filter tip (make sure there are no droplets left inside the tip, flick the tip inside the tube if necessary), place your samples in the freezer or proceed to the next

Anexo IV: Ata da aula de qualificação

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

DIVISÃO DOS CURSOS DE PÓS-GRADUAÇÃO

AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): **DIRCE LEIMI KOMURA**
Curso: BOTÂNICA
Nível: Doutorado
Orientador(a): Charles Eugene Zartman (INPA)

Título:

"DIVERSIDADE E ASPECTOS ECOLÓGICOS DOS MACROFUNGOS AGARICALES EM ÁREA DE CRONOSSEQUÊNCIA NA AMAZÔNIA CENTRAL"

BANCA JULGADORA:

TITULARES:

ANTONIO CARLOS WEBBER (UFAM)
MICHAEL JOHN GILBERT HOPKINS (INPA)
JOSÉ LUIS CAMARGO (INPA)
SÉRGIO PÉREZ GORJÓN (C.I.E.F.A.P(Argentina)
JEAN-MARC MONCALVO (University of Toronto, Canada)

SUPLENTES:

ANDRÉ LUIS WILLERDING (CBA)
ANI BEATRIZ J. MATSUURA (FIOCRUZ)

EXAMINADORES	PARECER	ASSINATURA
ANTONIO CARLOS WEBBER	(X) Aprovado ( ) Reprovado	
MICHAEL JOHN GILBERT HOPKINS	(X) Aprovado ( ) Reprovado	
JOSÉ LUIS CAMARGO	(X) Aprovado ( ) Reprovado	
SÉRGIO PÉREZ GORJÓN	(X) Aprovado ( ) Reprovado	
JEAN-MARC MONCALVO	(X) Aprovado ( ) Reprovado	

Manaus (AM), 16 de abril de 2012.

OBS: A AULA DA ESTUDANTE FOI CONSIDERADA ÓTIMA, MAS OS EXAMINADORES DETECTARAM PROBLEMAS NOS OBJETIVOS E NOS METODOS A SEREM EMPREGADOS RECOMENDAMOS QUE A ESTUDANTE FAÇA UMA READEQUAÇÃO DO PLANO PRINCIPALMENTE NO TOCANTE À CRONOSEQUENCIA POIS OS METODOS E AS PREMISSAS NÃO PARECEM ADEQUADOS

PROGRAMA DE PÓS-GRADUAÇÃO DO INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA
Av. André Araújo, 2936 – Bairro: Aleixo – Caixa Postal: 478 – CEP: 69.060-001, Manaus/AM.
Fone: (+55) 92 3643-3119 Fax:(+55) 92 3643-3119
site: http://pg.inpa.gov.br e-mail: cursobot@inpa.gov.br

Anexo V: Ata da defesa de Doutorado

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

GOVERNO FEDERAL
BRASIL
PAÍS RICO É PAÍS SEM POBREZA

Curso de Pós-Graduação em Botânica INPA

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos quatro dias do mês de março de 2016, às 08:30h, no auditório da biblioteca do INPA-Campus I, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Dr. Felipe Wartchow, Universidade Federal da Paraíba, Dra. Ruby Vargas Isla, Instituto Nacional de Pesquisas da Amazônia-INPA, Dra. Cristina Sayuri Maki, Universidade Federal do Amazonas- UFAM, Dr. Leandro Rafael de Assis, Instituto Nacional de Pesquisas da Amazônia-INPA/MAX PLANCK, e Dr. Mario Henrique Terra Araújo, Instituto Nacional de Pesquisas da Amazônia-INPA, tendo como suplentes: Dr. Alberto Vicentini, Instituto Nacional de Pesquisas da Amazônia-INPA, e Dr. Antonio Carlos Webber, Universidade Federal do Amazonas-UFAM, sob a presidência do primeiro, a fim de proceder a arguição pública de TESE DOUTORADO da discente, Dirce Leimi Komura, intitulada: "Macrofungos *Agaricales* em Áreas de Manejo Florestal na Amazônia Central" sob a orientação: Dr. Charles Eugene Zartman e co- orientação: Dr. Jean Marc-Moncalvo. Após a exposição, dentro do tempo regulamentar, o (a) discente foi arguido (a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

EXAMINADORES	PARECER		ASSINATURA
FELIPE WARTCHOW	(X) Aprovado	( ) Reprovado	Felipe Wartchow
RUBY VARGAS ISLA	(X) Aprovado	( ) Reprovado	Ruby Vargas Isla
CRISTINA SAYURI MAKI	(X) Aprovado	( ) Reprovado	Cristina Sayuri Maki
RAFAEL LEANDRO DE ASSIS	(X) Aprovado	( ) Reprovado	Rafael de Assis
MARIO HENRIQUE TERRA ARAÚJO	(X) Aprovado	( ) Reprovado	Mario Araújo
ALBERTO VICENTINI	( ) Aprovado	( ) Reprovado	
ANTONIO CARLOS WEBBER	( ) Aprovado	( ) Reprovado	

Manaus (AM), 04 de março de 2016.

OBS: Como sugestão fica a inclusão dos comentários feitos pela banca.

Nada mais havendo, foi lavrado a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Dr. Michael John Gilbert Hopkins
Coordenador do Programa de Pós Graduação em Botânica
PO. 258/ 2014