

The background is a dense, misty forest with tall trees and lush green foliage. In the foreground, several mushrooms are visible: a large white mushroom with a brown cap on the left, a smaller white mushroom with a brown cap in the center, and a bright yellow mushroom with a brown cap on the right. The text is overlaid on the image.

NELSON MENOLLI JUNIOR

*Amanitaceae e Pluteaceae em
áreas de Mata Atlântica da região
metropolitana de São Paulo, SP*

**SÃO PAULO
2009**

NELSON MENOLLI JUNIOR

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áreas de Mata Atlântica da região
metropolitana de São Paulo, SP

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

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Ficha Catalográfica elaborada pela Seção de Biblioteca do Instituto de Botânica

Menolli Junior, Nelson
M547a *Amanitaceae e Pluteaceae* em áreas de Mata Atlântica da região metropolitana de São Paulo, SP / Nelson Menolli Junior -- São Paulo, 2009.
125 p.il.

Dissertação (Mestrado) -- Instituto de Botânica da Secretaria de Estado do Meio Ambiente, 2009

Bibliografia.

1. Basidiomicetos. 2. Agaricales. 3. Taxonomia. I. Título

CDU : 582.284

À minha mãe Alda Cristina Facion Menolli e ao meu irmão Marcelo Menolli, meus alicerces, por todo amor, apoio e confiança durante esta etapa e em todos os momentos de minha vida.

AGRADECIMENTOS

Ao Instituto de Botânica e ao Programa de Pós-graduação em Biodiversidade Vegetal e Meio Ambiente pela infra-estrutura e suporte oferecidos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de mestrado.

À FAPESP pelo apoio financeiro ao projeto “*Basidiomycota (Agaricales e Aphylllophorales)* do Parque Estadual das Fontes do Ipiranga” (processo FAPESP 04/04319-2).

À minha orientadora Dra. Marina Capelari, pela constante confiança, compreensão, dedicação, postura profissional e imensurável apoio para realização deste trabalho. Muito obrigado!!!

À Dra. Luzia Doretto Paccola Meirelles, Universidade Estadual de Londrina, pelo apoio, confiança e suporte fundamentais para o início desta etapa.

À Dra. Noemia Kazue Ishikawa, Instituto Nacional de Pesquisas da Amazônia, por me abrir as portas para os estudos micológicos e pelo incentivo, amizade e acompanhamento durante as etapas de minha formação profissional.

À Dra. Marília Gaspar, Instituto de Botânica, pelo auxílio e disponibilização de equipamentos durante a amplificação das amostras de DNA.

À Dra. Maria Helena Pelegrinelli Fungaro e à MSc. Francine Matias de Paula, Universidade Estadual de Londrina, pelo seqüenciamento de algumas amostras de DNA.

Ao Dr. Tarciso de Sousa Filgueiras, Reserva Ecológica do IBGE, ao Dr. Jefferson Prado, Instituto de Botânica, e ao José Francisco Kuhar, FCEN Universidad de Buenos Aires, pelas sugestões e revisão das diagnoses em latim.

Ao Dr. Edison Paulo Chu, Instituto de Botânica, por toda colaboração e sugestões para realização deste trabalho.

À Dra. Ana Odete Santos Vieira, Universidade Estadual de Londrina, pela disponibilização e auxílio na busca de literatura.

Aos membros da banca do exame de qualificação, Dra. Rosely Ana Piccolo Grandi, Dra. Sandra Farto Botelho Trufem e Dra. Olga Yano, por toda contribuição.

Ao Klei Souza pelo excelente trabalho no preparo das ilustrações.

Aos curadores dos herbários DAOM, F, K e LIL pelo empréstimo dos materiais para estudo.

A todos os professores do Programa de Pós-graduação em Biodiversidade Vegetal e Meio Ambiente pelos conhecimentos transmitidos e a excelente formação proporcionada.

A todos os funcionários do Instituto de Botânica, em especial aos da Biblioteca e da Secretaria da Pós-graduação, por toda presteza e auxílio durante a realização deste trabalho.

Aos funcionários da Reserva Biológica de Paranapiacaba, em especial aos Srs. Antônio Dorival de Souza e Antonio Victor da Costa, pelo apoio durante as coletas.

Ao Dr. Clovis José Fernandes de Oliveira Júnior, à Dra. Rosângela Simão Bianchini e à Elizabete Aparecida Lopes pelo auxílio para a realização das coletas na Reserva Biológica de Paranapiacaba.

Aos funcionários do Parque Estadual da Cantareira, em especial à Sandra Bianchini e Adão Pereira Barbosa, pelo apoio durante as coletas na área.

To the great person Tatiane Asai, my right arm (and left!!!), you were essential. Thank you for your friendship, constant availability and patience during all the stages of this work. You are indispensable!

À Fernanda Karstedt, pela companhia e por todo apoio durante as coletas e viagens, além da disponibilização de fotos e auxílio durante as amostras para Microscopia Eletrônica de Varredura.

À Cássia A. Canavese e ao Luiz Antonio Silva Ramos pela agradável convivência no laboratório e durante as coletas. Ao Luiz, em especial, pela arte final da capa.

À minha prima Adriana Cristina Facion por toda paciência e constante disponibilidade para o preparo das pranchas do apêndice.

A todos os pesquisadores, funcionários e amigos da Seção de Micologia e Liquenologia pelos alegres momentos e valiosas contribuições.

Aos amigos e colegas responsáveis por bons momentos compartilhados durante a vivência em conjunto: Anderson Luiz dos Santos, Camila Francieli da Silva Malone, Carlos Eduardo Wetzel, Cristiane de Almeida Nascimento, Daniela Vinha, Diógina Barata, Érika Mattos Stein, Ewerton Caltran Manarin, Fernanda S. Lucas, Fernanda Tresmondi, Gisele Areia Nóbrega, Luciana Gomes Barbosa, Luciana Jandelli Gimenes, Maria Claudia M. P. de Medeiros, Rafael Batista Louzada, Sabrina Ribeiro Latansio Aidar e Thais Trindade de Lima.

Ao grande amigo Ewerton Caltran Manarin, por toda paciência, apoio e companheirismo tão importantes neste momento.

À minha grande amiga Esther do Lago e Pretti, companheira inseparável, presente em todos os momentos; à sua mãe Esther do Lago Pretti e a toda sua família pelo apoio e acolhimento desde minha chegada em São Paulo.

Aos grandes amigos Anderson Luiz dos Santos, Cristiane de Almeida Nascimento e Thais Trindade de Lima, responsáveis por momentos felizes e eternos. Thais, minha companheira favorita na cozinha; Ander, meu sempre prestativo amigo do coração; e Cris...“just” Cris!!!

À minha valiosa família por todo apoio: à minha mãe Alda Cristina Facion Menolli, por todo amor e pelo exemplo de vida e dedicação; ao meu pai Nelson Menolli, pela força e questionamentos científicos de grande valia; ao meu irmão Marcelo Menolli, pela confiança, compreensão, acolhimento e carinho; à minha irmã Simone Cristina Menolli Nascimento pela companhia, amizade e apoio. Amo vocês!!!

A todas as pessoas que, de alguma forma, contribuíram para a realização deste trabalho, minha eterna gratidão.

“Como seria o mundo sem fungos? Você provavelmente estaria acima das nuvens e embaixo de ti um oceano de galhos e folhas”.

João Vitor Campos e Silva

“Neste mundo que continua valendo a pena construir, as ciências e as artes são instrumentos e fruição, recursos para a vida e razão de vida”.

Luiz Carlos de Menezes

RESUMO

As famílias *Amanitaceae* e *Pluteaceae*, de conhecimento escasso para o Brasil, foram estudadas em três fragmentos de Mata Atlântica da região metropolitana do município de São Paulo. Foram realizadas coletas durante o período de doze meses, de janeiro a dezembro de 2007, no Parque Estadual da Cantareira (PEC), no Parque Estadual das Fontes do Ipiranga (PEFI) e na Reserva Biológica de Paranapiacaba (RBP). Materiais coletados antes ou após este período, bem como aqueles depositados no Herbário SP, também foram estudados. A descrição morfológica completa com as ilustrações para cada espécie é apresentada. Em adição, com o objetivo de estabelecer possíveis relações filogenéticas entre as espécies brasileiras dentro dos grupos estudados, foi conduzido um estudo molecular baseado em seqüências de ITS e nLSU do DNAr. *Amanita* foi o único gênero de *Amanitaceae* levantado no estudo, com a ocorrência de *A. coacta* para o PEFI. *Amanita coacta* é primeira citação para o sudeste brasileiro e são apresentadas informações morfológicas e moleculares que complementam a descrição original da espécie, juntamente com uma chave de identificação para as espécies de *Amanita* registradas para o Brasil. *Pluteaceae* foi representada nas áreas de estudo por *Pluteus*, presente nas três áreas, e por *Volvariella* limitada ao PEC. Para *Pluteus* são propostas quatro espécies novas (*P. aureovenatus*, *P. bulbomarginatus*, *P. capillicomptus* e *P. concavus*), uma variedade nova (*P. dominicanus* var. *hyalinus*) e o novo status de *P. sublaevigatus* para *P. chrysophlebius* subsp. *sublaevigatus*. *Pluteus fuligineovenosus*, *P. jamaicensis*, *P. riberaltensis* var. *conquistensis* e *P. longistriatus* são novas citações para o Brasil. Além disso, *P. umbrinoalbidus* é nova citação para o estado de São Paulo, *P. fluminensis* para o PEFI, *P. harrisii* para o PEC e para a RBP e *P. xylophilus* é ocorrência nova para o PEC. As análises de nLSU e ITS para as espécies de *Pluteus* revelaram um clado monofilético, com todas as espécies de *Pluteus*, dividido em dois clados, um com espécies da seção *Pluteus*, e outro incluindo os membros das seções *Hispidoderma* e *Celluloderma*. Para *Volvariella* foram descritas duas novas espécies, *V. heterospora* e *V. nullicystidiata*, e registradas *V. bombycina* e *V. perciliata*. Imagens de microscopia eletrônica de varredura e seqüências de DNA das espécies de *Volvariella* também foram disponibilizadas.

Palavras-chave: *Agaricales*, *Basidiomycota*, Taxonomia, Filogenia molecular

ABSTRACT

The families *Amanitaceae* and *Pluteaceae*, of scarce knowledge for Brazil, were studied in three Atlantic forest remnants of metropolitan region of São Paulo City. Collections were made for twelve months, from January to December 2007, at the Parque Estadual da Cantareira (PEC), Parque Estadual das Fontes do Ipiranga (PEFI) and Reserva Biológica de Paranapiacaba (RBP). The material collected before or after this period, as well as those deposited at herbaria SP, were also studied. The complete morphological description with illustrations for each species is presented. In addition, with the aim to establish possible phylogenetic relationships among the Brazilian species, a molecular study was performed based on sequences of nLSU and ITS rDNA. *Amanita* was the only genus of *Amanitaceae* surveyed on the study, with occurrence of *A. coacta* for PEFI. *Amanita coacta* is the first record for Southeast Brazil and were presented morphological and molecular data that complement its original description, a identification key of *Amanita* species recorded for Brazil is also presented. *Pluteaceae* was represented in the studied areas by *Pluteus*, occurring in the three areas, and *Volvariella* limited to the PEC. For *Pluteus* are proposed four new species (*P. aureovenatus*, *P. bulbomarginatus*, *P. capillicomptus* and *P. concavus*), a new variety (*P. dominicanus* var. *hyalinus*) and a new status from *P. sublaevigatus* to *P. chrysophlebius* subsp. *sublaevigatus*. *Pluteus fuligineovenosus*, *P. jamaicensis*, *P. riberaltensis* var. *conquistensis* and *P. longistriatus* are new records for Brazil. Moreover, *P. umbrinoalbidus* is a new record for São Paulo State, *P. fluminensis* for PEFI, *P. harrisii* for PEC and RBP and *P. xylophilus* for PEC. The nLSU and ITS analyses for *Pluteus* revealed a monophyletic clade with all *Pluteus* species divided in two clades, one with the species of section *Pluteus*, and another including members of both sections *Hispidoderma* and *Celluloderma*. For *Volvariella* were described two new species, *V. heterospora* and *V. nullicystidiata*, and recorded *V. bombycina* and *V. perciliata*. SEM (scanning electron microscopy) images and DNA sequences of *Volvariella* species were also disponibilized.

Palavras-chave: *Agaricales*, *Basidiomycota*, Taxonomy, Molecular phylogeny

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APRESENTAÇÃO

Este trabalho constitui um estudo taxonômico e molecular das espécies de *Amanitaceae* e *Pluteaceae* (*Agaricales*, *Basidiomycota*) coletadas em três remanescentes de Mata Atlântica na região metropolitana do município de São Paulo: Parque Estadual da Cantareira, Parque Estadual das Fontes do Ipiranga e Reserva Biológica de Paranapiacaba. Estas três áreas representam importantes remanescentes de Mata Atlântica em região urbana do estado de São Paulo e são locais de extremo interesse para o estudo da biodiversidade brasileira.

Os resultados do trabalho são apresentados em quatro capítulos, que correspondem a artigos, sendo um já publicado (capítulo IV), um aceito para publicação (capítulo I) e os outros dois (capítulos II e III) que ainda serão submetidos. Os artigos apresentados são precedidos de uma introdução geral sobre as famílias *Amanitaceae* e *Pluteaceae* com informações a respeito da taxonomia, filogenia molecular e distribuição geográfica do grupo.

O capítulo I apresenta a nova ocorrência de *Amanita coacta* Bas para o sudeste brasileiro, juntamente com informações morfológicas e moleculares que complementam a descrição original da espécie. Além disso, neste artigo, é apresentada uma chave de identificação para as espécies de *Amanita* registradas, em literatura, para o Brasil. Este artigo será publicado na revista *Mycotaxon* volume 107, 2009.

O capítulo II apresenta a descrição de quatro espécies de *Pluteus* coletadas no Parque Estadual da Cantareira e no Parque Estadual das Fontes do Ipiranga. Duas são espécies novas para a ciência; uma é citada pela primeira vez para o Brasil, e a restante é uma espécie com tipo brasileiro, conhecida até então somente pelo material tipo. Este artigo será submetido para publicação na revista *Mycologia*.

O capítulo III apresenta a descrição completa de dez espécies de *Pluteus* coletadas nas três áreas de estudo, junto com uma análise molecular do gênero, incluindo espécies brasileiras. No artigo, são propostas duas espécies novas, uma variedade nova e, ainda, três citações novas para o Brasil. Este artigo será submetido para publicação na revista *Mycological Research*.

O capítulo IV apresenta a descrição de quatro espécies de *Volvariella* coletadas no Parque Estadual da Cantareira, sendo que duas são espécies novas para a ciência. Em adição são disponibilizadas seqüências de genes de interesse na taxonomia e filogenia de basidiomicetos. Este artigo foi publicado na revista *Mycotaxon* volume 106, 2008.

Ao final é apresentada uma discussão geral dos resultados obtidos e, no apêndice, fotografias coloridas das espécies descritas no trabalho e que não estão disponíveis nos artigos.

Os epítetos das espécies novas descritas neste trabalho serão validamente publicados em outro local, de modo que esta publicação não deve ser considerada para efeitos taxonômicos, conforme permitido pelo artigo 34.1 do Código Internacional de Nomenclatura Botânica de Viena (McNeill *et al.* 2006). Tais nomes são apresentados aqui para melhor apresentação dos resultados e correta organização do texto.

As seqüências de DNA obtidas serão depositadas no GenBank quando da submissão dos artigos para publicação. Os capítulos de resultados estão formatados de acordo com as normas para publicação das revistas aos quais serão ou foram submetidos.

INTRODUÇÃO GERAL

1 CONSIDERAÇÕES GERAIS

A diversidade de fungos tem sido estimada por diversos autores, variando de 500 mil a 9,9 milhões de espécies, sendo que a estimativa mais aceita atualmente é de 1,5 milhões de espécies (Hawksworth 2001). Entretanto, apenas cerca de 97 mil estão descritas atualmente (Kirk *et al.* 2008), correspondendo a aproximadamente 6,4% do valor estimado. Deste modo, existe uma grande especulação em relação aos locais onde poderiam ocorrer os 1,4 milhões de fungos ainda não conhecidos (Hawksworth & Rossman 1997, Hyde 2001). Entre as diversas localidades possíveis de se encontrar esse imenso número de fungos ainda não explorados pela ciência, estão os países com conhecimento escasso de sua diversidade fúngica, e hospedeiros, habitats ou nichos inexplorados ou ainda pouco estudados como os ecossistemas tropicais (Hawksworth & Rossman 1997, Hyde 2001).

A diversidade de ecossistemas existente no Brasil, incluindo a Mata Atlântica e as florestas tropicais da Amazônia, contribui para a ocorrência de um grande número espécies de fungos macroscópicos. Os fungos com basidiomas macroscópicos pertencem, em sua maioria, à classe *Basidiomycetes* (*Basidiomycota*), sendo que aqueles comumente conhecidos como cogumelos e dotados de himênio lamelar, estão, em geral, incluídos na ordem *Agaricales* (Alexopoulos 1996, Gugliotta & Capelari 1998). Segundo Kirk *et al.* (2008) a ordem *Agaricales* consta de 13.233 espécies distribuídas em 33 famílias, o que corresponde a apenas cerca de 16,5% do número de espécies estimado para a ordem (Rossman 1994 *apud* Hawksworth 2001).

As famílias *Amanitaceae* R. Heim ex Pouzar e *Pluteaceae* Kotl. & Pouzar (*Agaricales*), de ampla distribuição mundial, com base em dados morfológicos e moleculares são consideradas relacionadas (Singer 1986, Moncalvo *et al.* 2002), e juntas compreendem aproximadamente 885 espécies (Kirk *et al.* 2008) distribuídas em seis gêneros, de acordo com a classificação de Singer (1986). Segundo Kirk *et al.* (2008) a família *Amanitaceae* compreende os gêneros *Amanita* Pers., *Catarrama* Franco-Mol. e *Limacella* Earle. Singer (1986) considerou em *Pluteaceae* os gêneros *Chamaeota* (W.G. Smith) Earle, *Pluteus* Fr. e *Volvariella* Speg. Kirk *et al.* (2008) considera quatro gêneros no verbete *Pluteaceae*, porém, o quarto gênero não foi encontrado na publicação. Até o momento, não há registros de *Chamaeota* para o Brasil, e, de maneira geral, ambas as famílias são pouco conhecidas e têm registros escassos para o Brasil e, em especial, para o estado de São Paulo.

A classificação da ordem *Agaricales* vem sendo drasticamente mudada nos últimos anos em função de análises de DNA em trabalhos de filogenia (Moncalvo *et al.* 2000b, 2002, Matheny *et al.* 2006) e mesmo de taxonomia (Vellinga *et al.* 2003, Lebel *et al.* 2004).

Os genes codificadores do RNA ribossomal e seus espaçadores são os marcadores moleculares mais comumente utilizados para a sistemática filogenética dos fungos, sendo muito úteis para a definição de gêneros e mesmo espécies novas (Lebel *et al.* 2004, Minnis *et al.* 2006, Lima *et al.* 2008). Entre estes marcadores, o gene nLSU (nuclear large subunit) do DNA ribossomal tem sido indicada para análises filogenéticas entre diferentes gêneros e espécies de fungos (Moncalvo *et al.* 2000b, 2002, Sjamsuridzal *et al.* 2002), sendo que a maioria das espécies pode ser diferenciada através do seqüenciamento destes marcadores. Já as regiões ITS1 e ITS2 (internal transcribed spacer) são mais utilizadas para distinguir espécies mais proximamente relacionadas ou mesmo populações (Lim & Jung 1998, Moreno *et al.* 2008, Lima *et al.* 2008).

Os estudos moleculares envolvendo as famílias *Amanitaceae* e *Pluteaceae* não incluem espécies tropicais e estão, basicamente, restritos à *Amanita*, incluindo diversos estudos filogenéticos para a compreensão da classificação infragenérica do gênero (Weiß *et al.* 1998, Drehmel *et al.* 1999, Moncalvo *et al.* 2000a, Oda *et al.* 1999, Zhang *et al.* 2004). Para os demais gêneros, o uso de métodos moleculares é restrito a trabalhos amplos que envolvem a circunscrição de grupos dentro de *Agaricales* (Moncalvo *et al.* 2000b, 2002, Matheny *et al.* 2006).

Buscando contribuir para o conhecimento das espécies de *Amanitaceae* e *Pluteaceae* no Brasil, o propósito deste trabalho foi efetuar o levantamento destas famílias em três remanescentes de Mata Atlântica (Parque Estadual da Cantareira, Parque Estadual das Fontes do Ipiranga e Reserva Biológica de Paranapiacaba) localizados na região metropolitana do município de São Paulo. O estudo foi feito com material depositado em herbários e coletas que foram realizadas mensalmente durante o período de um ano (01.2007–12.2007), também com o objetivo de estabelecer possíveis relações filogenéticas entre as espécies brasileiras dentro dos grupos estudados, com estudos moleculares a partir da obtenção de seqüências de genes de interesse na taxonomia e filogenia de basidiomicetos.

2 *Amanitaceae*

As espécies de *Amanitaceae* são caracterizadas por possuírem basidiomas carnosos, médios a grandes, pluteóides ou lepiotóides. Têm ampla distribuição mundial, são terrestres ou húmicas e freqüentemente estabelecem associações ectomicorrízicas. Possuem lamelas

livres ou sublivres, com estipe central bem desenvolvido e desenvolvimento bivelangiocárpico, com véu universal e parcial presentes. A presença do véu universal pode ser reconhecida pelas escamas na superfície do píleo e a presença de volva, ou em algumas espécies, o véu universal é reduzido a uma bainha mucilaginosa; enquanto que o véu parcial é frequentemente persistente como anel no estipe, podendo desprender-se ou estar ausente na maturidade. O contexto do píleo e do estipe é constituído de hifas inamilóides, de parede fina, com ou sem ansas, e com os elementos terminais frequentemente inflados (acrofisálides), e a trama do himênio tem organização bilateral divergente. Os cistídios são ausentes e os basidiósporos em geral são globosos a cilíndricos, hialinos, de parede fina, lisos ou raramente pouco ornamentados, amilóides ou inamilóides, acianófilos, estando presente em números de dois ou quatro por basídios (Pegler 1983, Singer 1986).

Atualmente, *Amanitaceae* compreende os gêneros *Amanita*, *Catatrama* e *Limacella* (Kirk *et al.* 2008). Porém, tradicionalmente ainda é adotada a delimitação de família como proposta por Singer (1986), onde *Amanitaceae* compreende apenas os gêneros *Amanita* e *Limacella*. A inclusão de *Catatrama*, originalmente descrita em *Tricholomataceae* (Franco-Molano 1991), foi recentemente proposta por Kirk *et al.* (2008), provavelmente com base nos dados moleculares apresentados por Moncalvo *et al.* (2002).

Estudos moleculares recentes (Moncalvo *et al.* 2000b, 2002, Matheny *et al.* 2006) demonstraram que os membros de *Amanitaceae* têm origem monofilética e estão intimamente relacionados com os membros de *Pluteaceae*. Esta relação entre as duas famílias já foi responsável pela classificação dos membros de *Amanitaceae* em *Pluteaceae* (Kirk *et al.* 2001), ou ainda pela inclusão do gênero *Pluteus* (*Pluteaceae*) na família *Amanitaceae* (Pegler 1977, Singer 1949).

2.1 *Amanita*

Amanita foi proposto por Persoon (1797) a partir da segregação de espécies, que continham volva membranosa ou friável, do gênero *Agaricus* L. A separação de espécies contendo volva feita por Persoon (1797) incluía algumas com basidiósporos rosados, que, posteriormente, foram então separadas por Fries (1821) e hoje constituem o gênero *Volvariella* (Bas 1969). A partir de então, baseado em diferenças como presença ou ausência de anel e variações morfológicas da volva, outros gêneros foram propostos: *Amanitaria* E.-J. Gilbert, *Amanitella* Earle, *Amanitina* E.-J. Gilbert, *Amanitopsis* Roze, *Amidella* E.-J. Gilbert, *Amplariella* E.-J. Gilbert, *Ariella* E.-J. Gilbert, *Aspidella* E.-J. Gilbert, *Lepidella* E.-J. Gilbert,

Leucomyces Earle, *Pseudofarinaceus* Kuntze, *Vaginata* Gray, *Venenarius* Earle. No entanto, hoje todos são considerados sinônimos de *Amanita* (Bas 1969, Pegler 1983).

Atualmente *Amanita* compreende cerca de 500 espécies (Kirk *et al.* 2008) e inclui espécies comestíveis, tóxicas ou mortais, caracterizadas, principalmente, pelo hábito usualmente ectomicorrízico obrigatório; véu universal bem desenvolvido, que primeiro envolve todo o basidioma e posteriormente fica reduzido a uma volva, ou como remanescentes, na forma de escamas, na superfície do píleo; basidiósporos amilóides ou inamilóides; margem da lamela estéril; hifas com, ou mais frequentemente, sem ansas; acrofisáides dispostas longitudinalmente compondo o contexto do estipe (Bas 1969, Pegler 1983, Singer 1986).

2.1.1 Classificação infragenérica

Com base na reação de amiloidia dos basidiósporos, o gênero *Amanita*, segundo Corner & Bas (1962) e Bas (1969), é dividido em dois subgêneros: *Amanita* caracterizado por apresentar basidiósporos inamilóides e *Lepidella* (E.-J. Gilbert) Veselý caracterizado por apresentar basidiósporos amilóides. Esta divisão em dois subgêneros é amplamente aceita e, nos últimos anos, tem sido alvo de estudos moleculares baseados em seqüências de ITS e nLSU que, em geral, têm reconhecido os dois subgêneros, seja com alto suporte (Drehmel *et al.* 1999, Oda *et al.* 1999) ou não bem suportados (Weiß *et al.* 1998, Zhang *et al.* 2004).

Corner & Bas (1962) e Bas (1969) também propuseram a subdivisão dos subgêneros em seções, com base nas diferenças morfológicas do estipe, volva, anel, margem e coloração do píleo. Desta forma, o subgênero *Amanita* foi subdividido em duas seções [*Amanita* e *Vaginatae* (Fr.) Quél.] e o subgênero *Lepidella* em quatro seções [*Amidella* (E.-J. Gilbert) Konr. & Maubl., *Lepidella* E.-J. Gilbert, *Phalloideae* (Fr.) Quél. e *Validae* (Fr.) Quél.].

Uma outra classificação em nível de seção foi proposta por Singer (1986) que dividiu o subgênero *Amanita* em quatro seções (*Amanita*, *Caesareae* Singer, *Ovigerae* Singer, e *Vaginata*), e o subgênero *Lepidella* em cinco seções [*Amidellae*, *Mappae* (E.-J. Gilbert), *Phalloideae*, *Roanokenses* Singer, e *Validae*]. Oda *et al.* (1999) propuseram um quadro comparativo, apresentado na Figura 1, que sintetiza estas duas classificações infragenéricas propostas para *Amanita*.

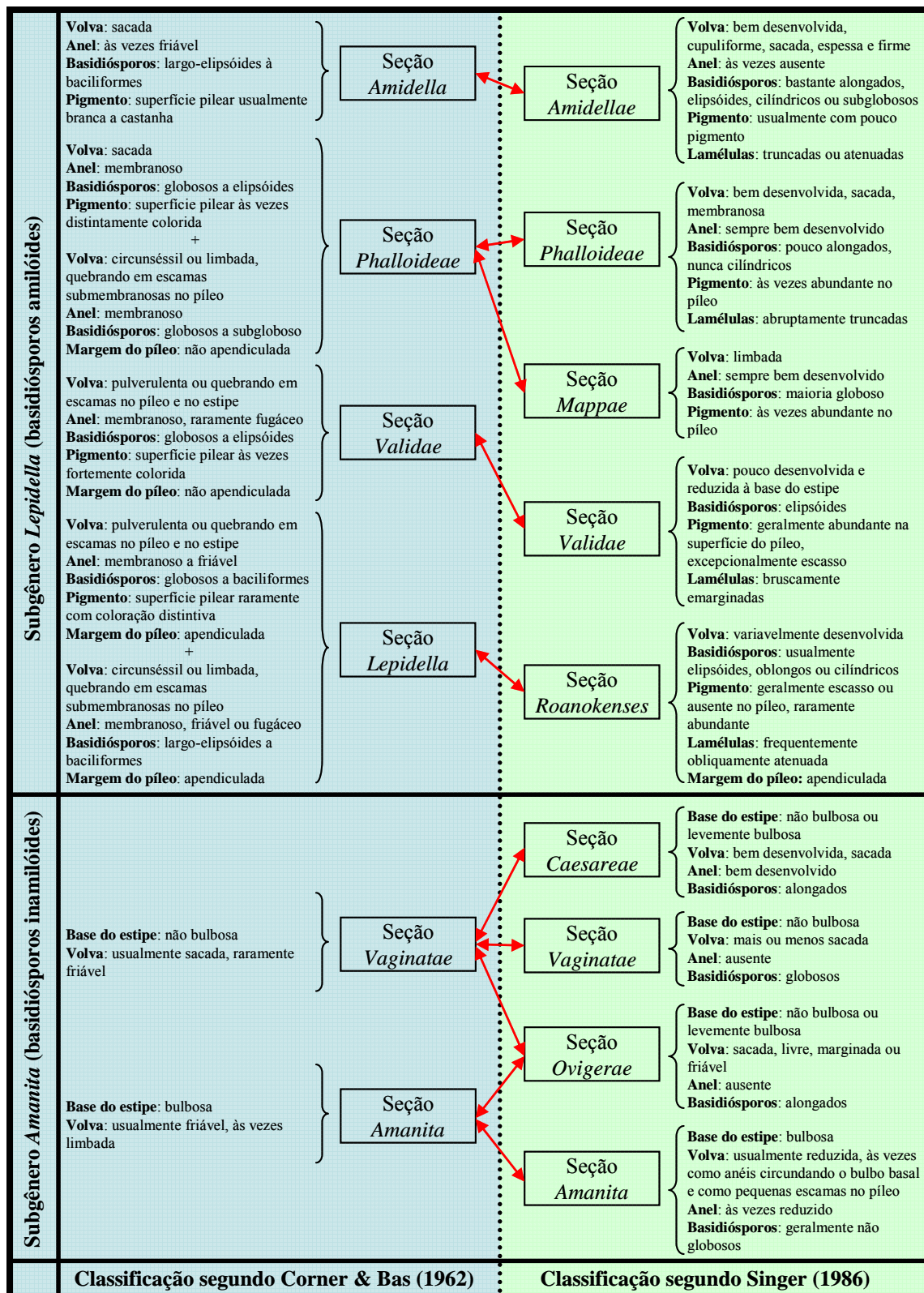


Figura 1: Comparação entre as classificações de *Amanita* propostas por Corner & Bas (1962) e Singer (1986). Adaptado de Oda *et al.* (1999).

Estudos moleculares reconhecem a origem monofilética de algumas das seções e têm dado suporte para as classificações propostas por Corner & Bas (1962) e Bas (1969) ou Singer (1986). Dentre estes, Zhang *et al.* (2004) demonstraram resultados de análises de

máxima parcimônia de nLSU que reconheceram as seções *Amanita*, *Vaginatae* e *Caesareae* para o subgênero *Amanita*, e as seções *Amidella*, *Lepidella*, *Phalloideae* e *Validae* para o subgênero *Lepidella*. Provavelmente, o reconhecimento destas seções como demonstrado por Zhang *et al.* (2004) reflete um consenso entre as duas classificações morfológicas. Resultados semelhantes foram também demonstrados por Weiß *et al.* (1998).

2.1.2 *Amanita* no Brasil

A partir de dados disponíveis na literatura, 19 taxóons de *Amanita* foram reportadas para o Brasil, com distribuição limitada a sete estados. Dentre estes, alguns são espécies exóticas, provavelmente introduzidas com mudas, como *A. muscaria* (L.) Lam. reportada para os estados do Paraná, Rio Grande do Sul, Santa Catarina e São Paulo (Homrich 1965, Figueiredo *et al.* 1996, Giachini *et al.* 2000, 2004, Meijer 2001, 2006, Sobestiansky 2005), *A. pantherina* var. *multisquamosa* (Peck) Jenkins reportada para Santa Catarina (Giachini *et al.* 2000, 2004), e *A. rubescens* Pers. reportada para o Rio Grande do Sul (Sobestiansky 2005).

Rick (1906) foi o primeiro autor a mencionar espécies de *Amanita* para o Brasil, com o registro de *A. spissa* (Fr.) P. Kummer e a descrição de duas variedades, *A. spissa* var. *alba* Rick [nom. illegit., non Qué.] e *A. spissa* var. *laeta* Rick, para o Rio Grande do Sul. Posteriormente, Rick (1930, 1937, 1961) citou *A. strobiliformis* (Paulet ex Vittad.) Bertill. e *A. bresadolae* (Rick) Rick [nom. illegit., non Schulzer], ambas para o Rio Grande do Sul. Singer (1953), revisando as espécies descritas por Rick, renomeou *A. bresadolae* como *Lepiota crassior* Singer, mas não fez nenhum comentário sobre as variedades de *A. spissa* publicadas por Rick (1906). Bas & Meijer (1993) consideraram *A. spissa* var. *laeta* como um possível sinônimo de *A. grillipes* Bas & de Meijer descrita para o Paraná.

Trabalhos subseqüentes às obras de Rick (1906, 1930, 1937, 1961) para o Rio Grande do Sul, também contribuíram para o conhecimento do gênero no Brasil, e, sem dúvida, a maior contribuição é representada pelo trabalho de Bas (1978) que descreveu oito espécies novas para o Amazonas: *A. campinaranae* Bas, *A. coacta* Bas, *A. craseoderma* Bas, *A. crebresulcata* Bas, *A. lanivolve* Bas, *A. phaea* Bas (nom. prov.), *A. sulcatissima* Bas e *A. xerocybe* Bas. Destas novas espécies descritas, apenas duas foram recoletadas em outros estados brasileiros: *A. craseoderma* em Rondônia (Capelari & Maziero 1988) e *A. crebresulcata* no Paraná (Meijer 2006), Pernambuco (Wartchow & Maia 2007) e São Paulo (Grandi *et al.* 1984, Pegler 1997).

Outros registros de *Amanita* para o Brasil incluem os trabalhos de Pegler (1997) que mencionou *A. ameghinoi* (Speg.) Singer para São Paulo; Meijer (2006) com o registro de

A. chrysoleuca Pegler para o Paraná e Wartchow *et al.* (2007b) com *A. lilloi* Singer para Pernambuco.

2.2 *Catatrama*

O gênero *Catatrama* foi originalmente descrito na família *Tricholomataceae* (Franco-Molano 1991) a partir de duas coleções provenientes da Costa Rica. Trata-se, até então, de um gênero monoespecífico que inclui apenas *C. costaricensis* Franco-Mol.

Franco-Molano (1991) ressaltou que, apesar do gênero ter sido descrito em *Tricholomataceae*, algumas características macro e microscópicas são compartilhadas com gêneros de *Agaricaceae* e *Amanitaceae*.

Catatrama costaricensis é caracterizada por apresentar hábito lepiotóide; lamelas brancas e sublivres; anel fibriloso presente no estipe; basidiósporos globosos, ornamentados, inamilóides e cianófilos; cistídios ausentes; trama do himênio bilateral divergente; e ansas sempre presentes. Apesar de apresentar estruturas típicas de *Amanitaceae* como lamelas livres a sublivres e trama do himênio bilateral divergente, *C. costaricensis* não apresenta acrofisálides e possui basidiósporos cianófilos (Franco-Molano 1991), características que não estão presentes na delimitação de *Amanitaceae* como proposta por Singer (1986).

Apesar das distinções morfológicas existentes para a inclusão de *Catatrama* em *Amanitaceae*, Moncalvo *et al.* (2002) demonstraram, com análise de parcimônia de nLSU, o agrupamento de *Catatrama* no clado de *Amanitaceae* e sua relação molecular com *Amanita* e *Limacella*, suportada pelos caracteres anteriormente apontados por Franco-Molano (1991). Atualmente, Kirk *et al.* (2008) aceitam *Catatrama* como um gênero de *Amanitaceae*, representado por uma única espécie, *C. costaricensis*, com distribuição geográfica limitada para a Costa Rica (Franco-Molano 1991), Índia (Vrinda *et al.* 2000) e recentemente para o estado de Pernambuco, Brasil (Wartchow *et al.* 2007a).

2.3 *Limacella*

Limacella é um gênero ainda pouco conhecido, com aproximadamente 20 espécies descritas (Kirk *et al.* 2008) e, provavelmente, com distribuição mundial, porém com a maior parte das espécies conhecidas para a América do Norte (Singer 1986).

As espécies do gênero descrito por Earle (1909) são caracterizadas por apresentarem hábito não obrigatoriamente ectomicorrízico; píleo mais ou menos viscoso, sem fragmentos de volva; volva sempre ausente ou representada por uma camada viscosa sobre o píleo; véu

parcial presente como uma camada gelatinosa ou como um anel membranoso; estipe seco ou viscoso; margem da lamela estéril; basidiósporos inamilóides e ansas presentes (Pegler 1983, Singer 1986).

De acordo com Pegler (1983) e Singer (1986) a classificação infragenérica de *Limacella* restringe-se à divisão em duas seções baseadas na viscosidade do estipe. Desta forma, espécies que apresentam estipe seco estão inclusas na seção *Limacella*, enquanto aquelas com estipe viscoso pertencem à seção *Lubricae* H.V. Sm.

Trabalhos de filogenia molecular envolvendo espécies de *Limacella* (Moncalvo *et al.* 2000b, 2002) apenas relacionam algumas espécies com outros gêneros e dão suporte para a classificação do gênero na família *Amanitaceae*, porém, sem nenhuma menção à classificação infragenérica adotada por Pegler (1983) e Singer (1986).

O conhecimento de *Limacella* para o Brasil é limitado ao registro de *Limacella guttata* (Pers.) Konrad & Maubl. para o estado de São Paulo (Município de Cananéia, Ilha do Cardoso) por Pegler (1997). Porém, não é apresentada uma descrição completa que confirme a ocorrência de *Limacella* para o Brasil.

3 *Pluteaceae*

Os representantes de *Pluteaceae* apresentam ampla distribuição mundial e podem ser terrestres, húmicas, lignícolas, coprófilos ou raramente micoparasitas, porém, nunca ectomicorrízicos. São caracterizados por possuírem basidiomas pluteóides, carnosos ou membranosos, com ampla variação de tamanho, desde pequenos a muito grandes, com lamelas livres e rosadas, e estipe central bem desenvolvido. Volva e anel podem estar presentes ou ausentes. A trama do himênio é bilateral convergente e as ansas podem estar presentes ou ausentes. Os cistídios estão sempre presentes e são característicos, podendo ser metulóides ou não, e os basidiósporos são globosos a elípticos, de parede espessada, lisos, inamilóides, cianófilos e caracterizados por uma coloração rosada percebida através da esporada (Pegler 1983, Orton 1986, Singer 1986).

De acordo com a classificação adotada por Pegler (1983) e Singer (1986), *Pluteaceae* é constituída de três gêneros: *Chamaeota*, *Pluteus* e *Volvariella* Sp. que juntos totalizam cerca de 359 espécies (Kirk *et al.* 2008).

Pluteaceae é bastante similar a *Amanitaceae*, diferindo principalmente pela forma inversa da trama da lamela, por possuir basidiósporos de estrutura, forma e parede mais complexas, além de não possuir hábito ectomicorrízico (Singer 1986). Esta relação entre *Pluteaceae* e *Amanitaceae* apontada por Singer (1986) vem sendo comprovada com dados

moleculares (Moncalvo *et al.* 2000b, 2002, Matheny *et al.* 2006) que mostram as duas famílias como grupo-irmãos e com origem monofilética, apesar do baixo suporte evidenciado.

3.1 *Chamaeota*

Chamaeota é um gênero pouco conhecido com cerca de dez espécies (Minnis *et al.* 2006, Kirk *et al.* 2008) e, provavelmente, com distribuição mundial. Para o Brasil não há registro de *Chamaeota*.

As espécies de *Chamaeota* compartilham características comuns de *Pluteaceae*, como lamelas livres e rosadas, basidiósporos de parede espessada e trama da lamela bilateral convergente (Pegler 1983, Minnis *et al.* 2006). No entanto, a ausência de volva e a presença de anel no estipe são características que delimitam e distinguem, de forma simples e conveniente, *Chamaeota* dos demais gêneros de *Pluteaceae* (Singer 1986).

Entretanto, estudos recentes (Minnis *et al.* 2006), envolvendo análises moleculares, têm demonstrado que espécies anteriormente classificadas como *Chamaeota* podem, na verdade, ser espécies de *Pluteus*. Minnis *et al.* (2006), a partir de análises de parcimônia de nLSU, propuseram a combinação de *Chamaeota mammilata* (Longyear) Murrill no gênero *Pluteus* já que, de acordo com as análises moleculares, esta espécie está inserida em um clado junto com outras espécies de *Pluteus*.

Esta relação entre *Chamaeota* e *Pluteus*, bem como a delimitação de *Chamaeota* com base na ausência de anel, já foi discutida anteriormente por Singer (1986), que ressaltou a impossibilidade, até aquele momento, de confirmar a distinção de um gênero com base apenas na ausência de anel, podendo esta característica ser a base de uma classificação artificial e conveniente para delimitação de *Chamaeota*. Singer (1986) ainda discutiu as similaridades compartilhadas entre a organização estrutural da superfície pilear de *Chamaeota* com as espécies de *Pluteus* seção *Hispidoderma*. Minnis *et al.* (2006) também sugeriram que outras espécies de *Chamaeota*, incluindo *C. tropica* Pegler e *C. subolivascens* Courtec., podem estar relacionadas às espécies de *Pluteus* seção *Celluloderma* por compartilharem o mesmo tipo de superfície pilear. Os autores acreditam ainda que o véu parcial possa ter surgido mais de uma vez no gênero, de forma que futuros estudos poderão demonstrar a possibilidade de *Chamaeota* ser sinônimo de *Pluteus*.

3.2 *Pluteus*

Pluteus é o mais representativo gênero de *Pluteaceae* com aproximadamente 300 espécies (Kirk *et al.* 2008) de ampla distribuição mundial e com hábito preponderantemente lignícola, crescendo frequentemente sobre madeira em decomposição, porém, às vezes húmicas ou terrestres (Pegler 1983, Orton 1986, Singer 1986). *Pluteus* difere dos demais gêneros de *Pluteaceae* basicamente pela ausência de volva e anel (Orton 1986). Entretanto, possui ainda outras características distintivas e úteis para a classificação infragenérica, como a presença de cistídios metulóides frequentemente com prolongamentos apicais e a organização da superfície pilear constituída por elementos globosos ou por hifas alongadas (Singer 1986).

A delimitação de *Pluteus* baseada na ausência de volva e anel vem sendo questionada nos últimos anos, já que, a partir de análises envolvendo biologia molecular (Minnis *et al.* 2006), tem-se verificado que espécies de *Chamaeota* (gênero de *Pluteaceae* com anel) agrupam em um mesmo clado com espécies de *Pluteus*. Já a relação de *Pluteus* com *Volvariella* (gênero de *Pluteaceae* com volva) só foi evidenciada por Singer (1958) que mencionou a presença de uma possível volva rudimentar em *P. stephanobasis* Singer. Entretanto, esta possível sobreposição de *Pluteus* e *Volvariella* não tem sido questionada, já que, trabalhos envolvendo análises moleculares têm usado com sucesso espécies de *Volvariella* como grupo externo de *Pluteus* (Minnis *et al.* 2006) e têm demonstrado a posição de *Volvariella* em um ramo separado de *Pluteus*, apesar da relação e origem monofilética dos dois gêneros (Matheny *et al.* 2006).

3.2.1 Classificação infragenérica

A classificação infragenérica mais aceita para *Pluteus* foi proposta por Singer (1958, 1986). Esta classificação divide o gênero em três seções (*Pluteus* Fr., *Hispidoderma* Fayod e *Celluloderma* Fayod) com base em características morfológicas, como a estrutura da superfície pilear e características dos pleurocistídios (Singer 1958, 1986). Desta forma, uma camada himeniforme de células globosas ou subglobosas caracteriza a superfície pilear da seção *Celluloderma*, enquanto que as outras duas seções exibem a superfície pilear com hifas alongadas, dispostas de forma ereta, subereta ou prostradas e diferem entre si pela presença de pleurocistídios metulóides na seção *Pluteus* ou de parede fina na seção *Hispidoderma* (Singer 1958, 1986). A seção *Celluloderma*, por sua vez, é dividida em duas subseções: subseção *Eucellulodermini* Singer que apresenta a superfície pilear com elementos uniformes e

dermatocistídios ausentes, e subseção *Mixtini* Singer que possui dermatocistídios e elementos dimórficos constituindo a superfície pilear (Singer 1958, 1986).

Outra classificação infragenérica também bastante difundida, porém sem grande aceitação, foi proposta por Vellinga & Schreurs (1985). Nesta classificação os autores propuseram a divisão do gênero em três seções também com base na estrutura da superfície pilear e nas características dos pleurocistídios. Duas das três seções possuem espécies com superfície pilear constituída por hifas prostradas ou com os elementos terminais ascendentes: a seção *Pluteus*, mantida de acordo com classificação de Singer (1958, 1986), que inclui espécies com pleurocistídios metulóides e a seção *Villosi* Schreus & Vellinga, proposta para aquelas espécies com pleurocistídios ausentes, ou se presentes de parede fina e sem prolongamentos apicais. A terceira seção proposta foi *Celluloderma* que inclui três subseções e engloba uma gama maior de variações da superfície pilear quando comparada à classificação de Singer (1958, 1986). As espécies da seção *Celluloderma*, de acordo com Vellinga & Schreurs (1985), possuem pleurocistídios de parede fina, superfície pilear tricodérmica, himeniforme ou intermediária entre himeniforme e epitelial, e foi subdividida em: subseção *Eucellulodermini*, que inclui espécies com a superfície pilear formada por elementos uniformes, esferopedunculados a clavados; subseção *Mixtini*, com superfície pilear constituída de elementos dimórficos com dermatocistídios; e subseção *Hispidodermini* (Fayod) Vellinga & Schreus, com superfície pilear tricodérmica ou himeniforme composta por elementos uniformes, cilíndricos a fusiformes.

Evidências moleculares para o suporte das classificações infragenéricas propostas estão restritas ao trabalho de Moncalvo *et al.* (2002) que demonstraram, a partir de análises de parcimônia de nLSU, a divisão das espécies de *Pluteus* em pelo menos dois clados com bom suporte, onde, um deles inclui as espécies da seção *Pluteus* e o segundo é composto por membros da seção *Hispidoderma* e *Celluloderma*.

3.2.2 *Pluteus* no Brasil

Hennings (1900) foi o primeiro autor a descrever espécies de *Pluteus* para o Brasil, com *P. scruposus* Henn. para o estado do Mato Grosso. Posteriormente, (Hennings 1904a, 1904b) descreveu *P. cervinus* var. *griseoviridis* Henn. para São Paulo (São Paulo, Serra da Cantareira) e *P. termitum* Henn. para o Amazonas. Entretanto, o material tipo destas espécies não foi preservado e provavelmente podem não representar espécies de *Pluteus* como mencionado por Saccardo & Trotter (1912), que reconheceram *P. termitum* como um possível sinônimo de *Collybia eurhiza* (Berk.) Höhn., e por Singer (1958) que estabeleceu

P. scruposus como sinônimo de *Oudemansiella canarii* (Jungh.) Höhn. e *P. termitum* como uma provável espécie de *Lepiota* (Pers.) Gray.

Rick (1907, 1919, 1930, 1938, 1961) também descreveu e mencionou algumas espécies duvidosas, totalizando 21 taxões de *Pluteus* registrados para o Rio Grande do Sul, incluindo seis espécies e duas variedades com tipos brasileiros [*P. cristatulus* Rick, *P. exiguus* var. *venosus* Rick, *P. fibrillosus* Rick, *P. leptonia* Rick, *P. nanus* var. *podospileus* (Sacc. & Cub.) Rick, *P. sensitivus* Rick, *P. straminellus* Rick e *P. velatus* Rick]. Entretanto, Singer (1953, 1958) considerou várias das coleções de J. Rick como identificações errôneas, *nomina dubia*, sinônimos ou espécies de outros gêneros.

Outras nove espécies com tipos brasileiros foram descritas para os estados do Amazonas, Rio de Janeiro, Rio Grande do Sul e Pará por Bresadola (1920) e Singer (1953, 1956, 1958, 1989): *P. amazonicus* Singer, *P. cervinus* var. *brasiliensis* Bres., *P. fluminensis* Singer, *P. hylaeicola* Singer, *P. melanopotamicus* Singer, *P. riograndensis* Singer, *P. subfibrillosus* Singer, *P. umbrinoalbidus* Singer e *P. varzeicola* Singer.

Singer (1956, 1989) mencionou também a ocorrência de *P. burserae* Singer e *P. minutus* Pat. para os estados de Rondônia e Bahia respectivamente. Grandi *et al.* (1984), Raithelhuber (1991), Stijve & Meijer (1993), Pegler (1997), Meijer (2001, 2006) e Drechsler-Santos *et al.* (2007) também contribuíram para o aumento do conhecimento de *Pluteus* no Brasil, com registros de espécies para o Paraná, São Paulo e Rio Grande do Sul. Porém, estes trabalhos apresentam apenas uma lista sem descrição completa das espécies.

Recentemente, Wartchow *et al.* (2004, 2006) forneceram descrições completas para *P. albostipitatus* (Dennis) Singer, *P. aquosus* Singer, *P. beniensis* Singer, *P. globiger* Singer, *P. nigrolineatus* Murrill e *P. thomsonii* (Berk. & Broome) Dennis coletados no Rio Grande do Sul. Entretanto, Rodríguez *et al.* (2008) consideraram que a espécie européia *P. thomsonii* mencionada por Wartchow *et al.* (2004) é, provavelmente, *P. neotropicalis* Rodr.-Alcánt., recentemente descrita (Rodríguez *et al.* 2008) com base em material mexicano anteriormente identificado como *P. thomsonii* (Rodríguez & Guzmán-Dávalos 1999).

3.3 *Volvariella*

Volvariella foi proposto por Spegazzini (1899) que o considerou diferente de *Volvaria* (Fr.) Kummer por ter o estipe cartilaginoso e contínuo com o píleo (Shaffer 1957). Entretanto, estas diferenças não justificaram o reconhecimento de um gênero distinto, e a espécie tipo designada para *Volvariella* seria na verdade uma espécie de *Volvaria* (Shaffer 1957). *Volvaria* foi considerada por Fries (1821) como uma tribo de *Agaricus* que incluía basidiomas com

volva, lamelas livres e basidiósporos rosados. Porém, *Volvaria* era um nome pré-ocupado pelo gênero de líquen *Volvaria* DC., e a conservação de *Volvaria* (Fr.) Kummer frente a *Volvaria* DC. não foi aceita (Shaffer 1957, Rogers 1953). Murrill (1911) propôs ainda o gênero *Volvariopsis* para as espécies com basidiósporos rosados, que foi estabelecido como sinônimo de *Volvariella* (Shaffer 1957).

Atualmente, *Volvariella* compreende cerca de 50 espécies (Kirk *et al.* 2008) e é facilmente reconhecido dentre os demais gêneros de *Pluteaceae* devido à presença de uma volva bem desenvolvida na base do estipe (Pegler 1983, Orton 1986, Singer 1986). Trata-se de um gênero cosmopolita, com espécies terrestres, lignícolas, coprófilas ou micoparasitas e, que em geral, apresentam superfície pilear constituída por hifas prostradas e sem ansas (Pegler 1983).

Os trabalhos de filogenia molecular envolvendo espécies de *Volvariella* estão restritos a trabalhos amplos, envolvendo vários grupos de *Agaricales* (Matheny *et al.* 2006), e apenas as relacionam com *Pluteus* e dão suporte para a classificação em *Pluteaceae*.

3.3.1 *Volvariella* no Brasil

A partir de dados disponíveis em literatura, 17 espécies de *Volvariella* foram reportadas para seis estados brasileiros (Menolli & Capelari 2008). Montagne (1856) foi o primeiro autor a mencionar *Volvariella* para o Brasil, com o registro de *V. cnemidophora* (Mont.) Singer (como *Agaricus cnemidophorus* Mont.). Rick (1930, 1961) mencionou oito espécies para o estado do Rio Grande do Sul: *V. bombycina* (Schaeff.) Singer [como *Volvaria bombycina* (Schaeff.) P. Kumm.], *V. cnemidophora* [como *Volvaria cnemidophora* (Mont.) Sacc.], *V. fibrillosa* (Bres. ex Rick) Singer (como *Volvaria fibrillosa* Bres.), *V. pusilla* (Pers.) Singer [como *Volvaria parvula* (Weinm.) P. Kumm.], *V. surrecta* (Knapp) Singer [como *Volvaria loveana* (Berk.) Gillet], *V. taylorii* (Berk. & Broome) Singer [como *Volvaria taylorii* (Berk. & Broome) Gillet], *V. thwaitesii* (Hook. f.) G.C. Rath [como *Volvaria thwaitesii* (Hook. f.) Sacc.] e *V. volvacea* (Bull.) Singer [como *Volvaria volvacea* (Bull.) P. Kumm.].

Para o estado de Pernambuco, Batista (1957) descreveu *V. oswaldoi* (Bat.) Putzke (como *Volvaria oswaldoi* Bat.) e mencionou *V. gloiocephala* (DC.) Boekhout & Enderle [como *Volvaria speciosa* (Fr.) P. Kumm.] e, posteriormente, *V. cnemidophora* foi mencionada por Batista & Bezerra (1960). Singer (1989) descreveu duas novas espécies, *V. macrospora* Singer e *V. rondoniensis* Singer, para o Amazonas e Rondônia respectivamente.

Para São Paulo há o registro de três espécies: *V. bakeri* (Murrill) Shaffer (Grandi *et al.* 1984, Pegler 1997), *V. bombycina* (Bononi *et al.* 1981) e *V. taylorii* (Pegler 1997). Meijer

(2001, 2006) mencionou dez espécies de *Volvariella* para o estado do Paraná, incluindo *V. bombycina*, *V. earlei* (Murrill) Shaffer, *V. gloiocephala*, *V. hypopithys* (Fr.) M.M. Moser, *V. cf. murinella* (Quél.) M.M. Moser, *V. perciliata*, *V. cf. pusilla* var. *taylorii* (Berk. & Broome) Boekhout, *V. rondoniensis*, *V. cf. striata* N.C. Pathak e *V. volvacea*.

ÁREA DE ESTUDO

1 CARACTERIZAÇÃO DA MATA ATLÂNTICA

As florestas tropicais úmidas ocupam 7% da superfície da terra e são consideradas os ambientes mais ricos em biodiversidade, abrigando mais de 50% do total das espécies nas terras emersas do planeta (Myers *et al.* 2000). A Mata Atlântica é a segunda maior floresta tropical do continente americano, que originalmente cobria mais de 1,5 milhões de km², sendo 92% dessa área distribuída ao longo da costa brasileira, desde o Rio Grande do Norte até o Rio Grande do Sul, e o restante penetrando até o leste do Paraguai e nordeste da Argentina em sua porção sul (Galindo-Leal & Câmara 2003).

Do ponto de vista legal, conforme define o Decreto Federal 750 de 1993, e, levando em consideração o tipo de solo, clima e relevo, a Mata Atlântica abrange as formações florestais e ecossistemas associados que incluem Floresta Ombrófila Densa, Floresta Ombrófila Mista, Floresta Ombrófila Aberta, Floresta Estacional Semidecidual e Brejos Orográficos (ou encaves florestais interioranos) no Nordeste (Guedes *et al.* 2005).

A área que compreende a Mata Atlântica varia em elevação altitudinal desde o nível do mar até 2.900 m (Mantovani 2003). Segundo a classificação técnica de Köppen, o clima ao longo da Mata Atlântica varia entre *Aw* (tropical), *Cwa* (tropical de altitude) e *Cf* (subtropical), com temperaturas médias entre 14-21°C, chegando à máxima absoluta de 35°C e não ultrapassando a mínima absoluta de 1°C. A pluviosidade anual média varia de 1.500 a 2.000 mm em função da região geográfica e, em particular, do relevo. Entretanto, devido, entre outros fatores, à ampla distribuição latitudinal deste bioma, estes dados fornecem apenas uma generalização geográfica sobre a abrangência da Mata Atlântica. Assim, em algumas áreas montanhosas de São Paulo a pluviosidade anual pode atingir índices excepcionalmente elevados, entre 3.600 e 4.500 mm, que representam os maiores valores para o Brasil, considerando inclusive os índices pluviométricos da floresta amazônica (Guedes *et al.* 2005).

Atualmente, a Mata Atlântica é um dos 34 *hotspots* mundiais, ou seja, centros de altíssima biodiversidade, incluindo pelo menos 1.500 espécies endêmicas de plantas, em que a extensão original foi reduzida em pelo menos 70%, colocando em risco a sobrevivência de incontáveis espécies (Mittermeier *et al.* 2004). Embora tenha sido em grande parte destruída, restando apenas cerca de 8% de sua extensão original (Figura 2), ela ainda abriga mais de 8.000 espécies endêmicas de plantas vasculares, anfíbios, répteis, aves e mamíferos (Fonseca *et al.* 2004, Mittermeier *et al.* 2004).



Figura 2: Vegetação original e remanescentes de Mata Atlântica no Brasil. Disponível em: <www.rbma.org.br>. Acesso em: 02 dez. 2008.

Neste sentido, a realização de estudos em remanescentes de Mata Atlântica é de grande importância, pois permite uma ampliação do conhecimento da biodiversidade que constitui este bioma. Ainda, a busca de novas espécies bem como o conhecimento da ocorrência de espécies já conhecidas em áreas pouco estudadas é fortemente embasada diante da grandeza e do valor da biodiversidade de ecossistemas tropicais e contribuem para o conhecimento e uso da biodiversidade de forma sustentável a fim de proteger legalmente os recursos naturais do país.

Na região metropolitana do município de São Paulo estão localizados alguns remanescentes de Mata Atlântica de grande importância para estudos de diversidade biológica, como o Parque Estadual das Fontes do Ipiranga (PEFI) localizado na região sul, o Parque Estadual da Cantareira (PEC) na região norte e a Reserva Biológica de Paranapiacaba (RBP) no município de Santo André (Figura 3), que foram escolhidos, neste trabalho, para os estudos de levantamento das espécies de *Amanitaceae* e *Pluteaceae*.

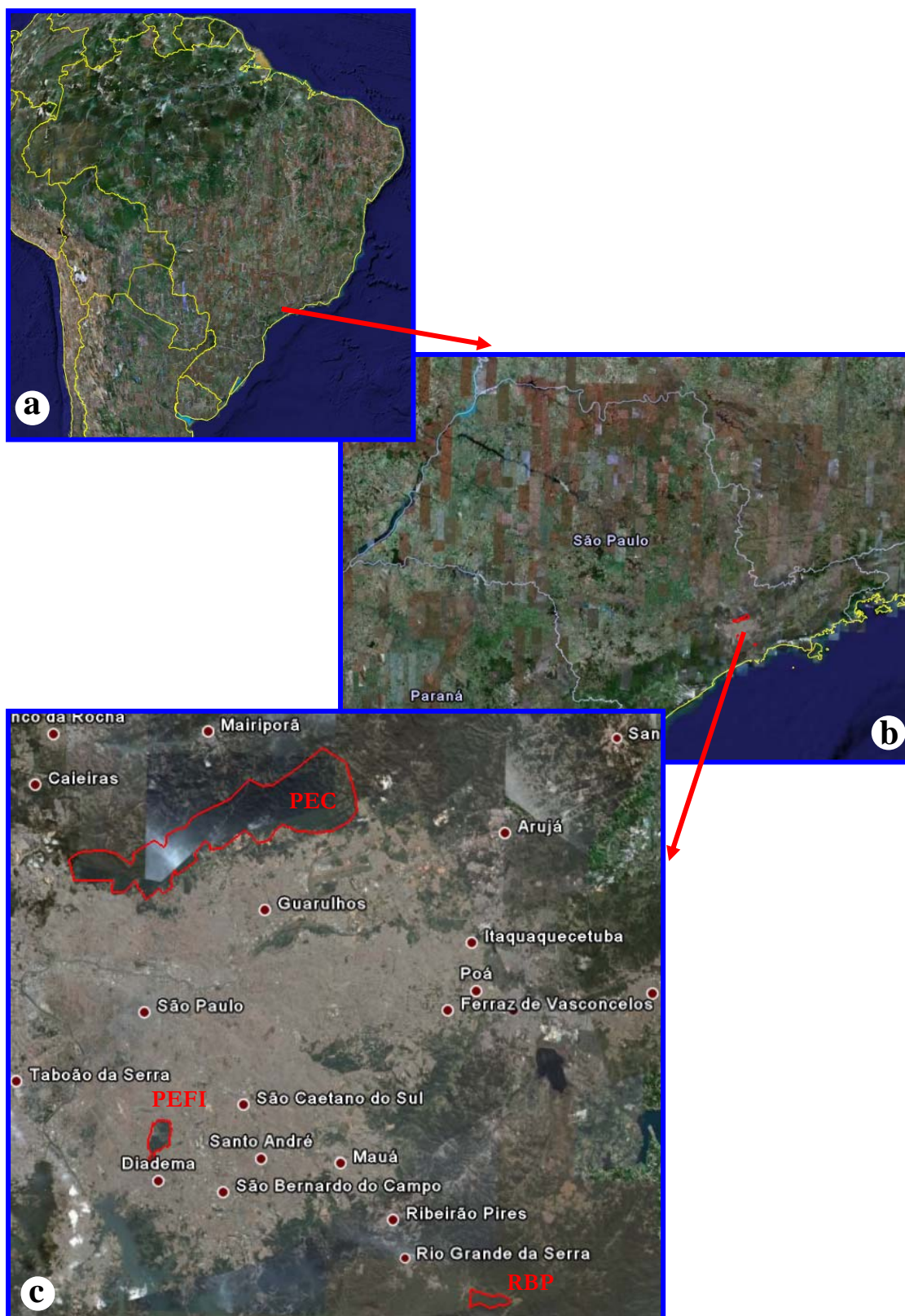


Figura 3: Localização das áreas de estudo: a. Brasil; b. Estado de São Paulo; c. Parque Estadual da Cantareira (PEC), Parque Estadual das Fontes do Ipiranga (PEFI), Reserva Biológica de Paranapiacaba (RBP). Imagens Google Earth 2007.

2 PARQUE ESTADUAL DA CANTAREIRA

O Parque Estadual da Cantareira (PEC) está situado no Planalto Atlântico (Figura 4), com altitudes que variam de 750 a 1.215 m, numa área de aproximadamente 7.900 ha (23°32'36"S e 46°37'59"W), e abrange os municípios de Caieiras, Guarulhos, Mariporã e extremo norte de São Paulo (Xavier *et al.* 2008, Secretaria do Meio Ambiente 2000).

Trata-se da maior unidade de conservação do mundo situada dentro de perímetro urbano, cuja vegetação pertence ao grupo de Floresta Estacional Semidecidual do Domínio da Mata Atlântica, e está sob influência de um clima mesotérmico úmido com temperatura média anual de 18,2°C para fevereiro, o mês mais quente, e de 14,3°C para julho, o mês mais frio (Instituto Florestal, Secretaria do Meio Ambiente 2000).

A área do PEC é dividida em quatro núcleos destinados para fins científicos, culturais, educativos e recreativos: Águas Claras, Cabuçu, Engordador e Pedra Grande. O núcleo Engordador (Figura 4), escolhido para os estudos de levantamento das espécies de *Amanitaceae* e *Pluteaceae*, juntamente com o núcleo Pedra Grande, são os dois núcleos com desenvolvimento de atividades de manejo, proteção e pesquisa, proporcionando estudos científicos sobre a biodiversidade, a fim de garantir a perpetuação das espécies que nela habitam (Instituto Florestal, Secretaria do Meio Ambiente 2000).



Figura 4: Área de Estudo: a. Parque Estadual da Cantareira (Imagem Google Earth 2007), * área de coleta (Núcleo Engordador); b. Aspecto da vegetação do PEC (Foto: N. Menolli Jr.).

3 PARQUE ESTADUAL DAS FONTES DO IPIRANGA

O Parque Estadual das Fontes do Ipiranga (PEFI), localizado na parte sudeste do município de São Paulo, SP, entre os paralelos 23°38'08''S e 23°40'18''S e os meridianos 46°36'48''W e 46°38'00''W (Fernandes *et al.* 2002), representa uma unidade de conservação que abriga a terceira maior reserva de Mata Atlântica da região metropolitana do município de São Paulo (Barros *et al.* 2002). Trata-se de um fragmento florestal situado em uma altitude média de 798 m e com área total de 543 ha (Xavier *et al.* 2008, Fernandes *et al.* 2002). As coletas dos representantes de *Amanitaceae* e *Pluteaceae* foram realizadas nas áreas de mata adjacentes ao Instituto de Botânica (Figura 5).

A vegetação do PEFI pertence ao grupo das Florestas Pluviais Tropicais do Domínio da Mata Atlântica e, mais especificamente, ao grupo das Florestas Estacionais Semidecíduais de Planalto (Pivello & Peccinini 2002). Trata-se de um dos poucos fragmentos remanescentes da Floresta Atlântica de Planalto, cujas áreas de florestas têm sido alvo de perturbações diretas e indiretas decorrentes tanto do processo de fragmentação e isolamento quanto das atividades humanas realizadas em seu entorno e em seu interior. Assim, o parque abriga hoje alguns tipos de florestas secundárias em aparente regeneração e também áreas florestais em provável degeneração (Pivello & Peccinini 2002).

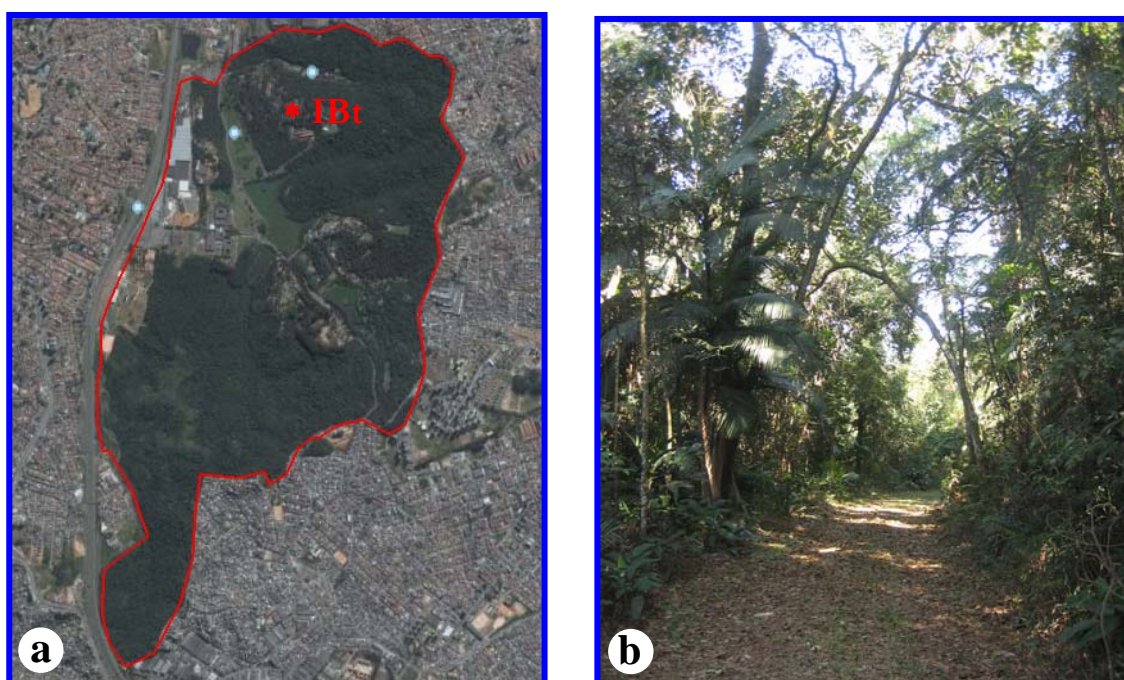


Figura 5: Área de Estudo: a. Parque Estadual das Fontes do Ipiranga (Imagem Google Earth 2007), * área de coleta, IBt = Instituto de Botânica; b. Aspecto geral da vegetação do PEFI (Foto: N. Menolli Jr.).

O clima na área do Parque, considerando sua posição junto à Bacia Hidrográfica do Alto Tietê, pode ser classificado como *Cwb* (Köppen), que significa clima temperado com regime de chuvas no verão e inverno seco (Santos & Funari 2002). Dados meteorológicos de 68 anos de estudos (1933-2000) dentro da área do PEFI demonstraram precipitação anual média de 1.368 mm, temperatura máxima absoluta de 35,6°C e maior média mensal de 23,9°C, e umidade relativa do ar com uma máxima absoluta de 100% e mínima absoluta de 13% (Santos & Funari 2002).

4 RESERVA BIOLÓGICA DE PARANAPIACABA

A Reserva Biológica de Paranapicaba (RBP) é de responsabilidade administrativa do Instituto de Botânica e está localizada em uma área de 336 ha no município de Santo André, SP (23°46'S e 46°18'W), na borda do Planalto Atlântico (Figura 6), a uma altitude entre 750-890 m (Xavier *et al.* 2008, Secretaria do Meio Ambiente 2000, Domingos *et al.* 2000). Nas últimas décadas, foram percebidas profundas alterações na estrutura da vegetação local, devido aos efeitos das, antes não controladas, emissões gasosas poluentes resultantes das atividades do complexo industrial de Cubatão, situado na planície abaixo da RBP (Secretaria do Meio Ambiente 2000).



Figura 6: Área de Estudo: a. Reserva Biológica do Alto da Serra de Paranapicaba (Imagem Google Earth 2007), b. Aspecto geral da vegetação da RBP (Foto: F. Karstedt).

A vegetação da RBP é caracterizada como uma Floresta Pluvial Tropical (Floresta Ombrófila Densa) Submontana do Domínio da Mata Atlântica, e a área apresenta um clima *Cfb* (Köppen), úmido temperado com ausência de estação seca, com média anual de temperatura de 17.9°C (média de 52 anos: 1870-1922), precipitação anual média de 3.381 mm (média de 23 anos: 1945-1968), e umidade relativa do ar 100% a maior parte do ano (Domingos *et al.* 2000).

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CAPÍTULO I

***Amanita coacta* (*Amanitaceae*, *Agaricales*) with a key
to species of *Amanita* occurring in Brazil***

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*Artigo aceito para publicação na revista *Mycotaxon* volume 107, 2009.

***Amanita coacta* (Amanitaceae, Agaricales) with a key to species of *Amanita* occurring in Brazil**

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Abstract — *Amanita coacta* was described from Amazonas State, Northern Brazil, in 1978 based on a single collection, and since then no more records have been reported. Sixteen collections of this species were made from Parque Estadual das Fontes do Ipiranga, São Paulo State, Southeast Brazil. These collections form a basis for a re-description including range variation of spore size and shape. In addition, an artificial dichotomous key to species of *Amanita* occurring in Brazil is presented.

Key words — *Basidiomycota*, Atlantic forest, taxonomy

Introduction

Amanita Pers. is a well-known genus of *Basidiomycota* with global distribution. It has morphological, anatomical and developmental characteristics useful for its macroscopical recognition and support inside the family *Amanitaceae* R. Heim ex Pouzar (Drehmel et al. 1999, Oda et al. 1999, Zhang et al. 2004). The genus is characterized by the mycorrhizal habit, hemiangiocarpic development, usually white and free lamellae, pallid basidiospores, bilateral hymenophoral trama and longitudinally acrophysalidic stipe tissue (Bas 1969).

The genus comprises ca. 500 species (Kirk et al. 2001) with 19 taxa mentioned for Brazil, including *A. muscaria* (L.) Lam. (Homrich 1965, Figueiredo et al. 1996, Fosco-Mucci & Yokomizo 1985, Giachini et al. 2000, 2004, Meijer 2001, 2006, Sobestiansky, 2005), *A. pantherina* var. *multisquamosa* (Peck) Jenkins (Giachini et al. 2000, 2004) and *A. rubescens* Pers. (Sobestiansky 2005), all of which were frequently collected in reforestation area with plantations of exotic trees and so likely introduced with seedlings.

Rick (1906) was the first author to describe species of *Amanita* from Brazil and, after that, other publications (Rick 1930, 1937, 1961, Singer 1953, Bas 1978, Grandi et al. 1984, Capelari & Maziero 1988, Bas & Meijer 1993, Pegler 1997, Wartchow et al. 2007, Wartchow & Maia 2007) also contributed to our knowledge of the genus in Brazil.

The study of Bas (1978), although restricted to the species collected by Rolf Singer in Amazonas State, Northern Brazil, is undoubtedly the best

contribution to knowledge about *Amanita* in Brazil. In his work, eight species with Brazilian types were described, including *A. coacta* based on a single collection from Amazon Forest.

In this article, *A. coacta* is reported for the first time outside the Amazon region, in a remnant of Atlantic forest of Southeast Brazil. This record represents the second collection of this species since its description. We provide an updated description of the species and presents for the first time photos of *A. coacta* showing different morphological patterns. In addition, an artificial dichotomous key for species of *Amanita* occurring in Brazil is also presented.

Material and methods

The specimens studied were collected at the Parque Estadual das Fontes do Ipiranga, a Atlantic forest remnant in São Paulo State, Southeast Brazil and deposited at herbarium SP. Fresh specimens were photographed and macro-morphological data were recorded. Color terms are according to Küppers (1979).

For microscopic analyses, the dried material was rehydrated in 70% ethanol followed by 5% KOH or Melzer's reagent. All microscopic illustrations were made with the aid of a drawing tube. The spores were measured in lateral view. The method for metric values follows Tulloss (1993). At the beginning of a set of spores data, the notation "[*a/b/c*]", where *a*, *b*, and *c*, are integers, is to be read "*a* spores were measured from *b* basidiomata taken from *c* collections". When ranges are provided in spore data in the form "*(m-n-o(-p))*", where *m*, *n*, *o* and *p* are integers, the values given are to be understood as follows: *m* is the lowest values observed or calculated and *p* the highest. In the range of values observed or calculated, the 5th percentile is *n* and the 95th percentile is *o*. A summary of definitions of biometric variables follows:

W_{cs} = breadth of central stratum of lamella.

$W_{st-near}$ = distance from one side of central stratum to nearest base of basidium.

W_{st-far} = distance from one side of central stratum to the most distant base of basidium on the same side of the central stratum.

L, (**W**) = the range of average lengths (widths) of spores of each basidioma examined.

L', (**W'**) = the average of all lengths (widths) of spores measured.

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

Q = the average of **Q** computed for all basidiomata examined.

Q' = the average of all **Q** values computed for all spores measured.

DNA sequences of the large subunit (LSU) of nuclear ribosomal DNA were obtained from two collections, for future phylogenetic molecular studies. GenBank accession numbers are cited below the species name at the beginning of the formal description. The dichotomous key presented is based on literature. Generic and infrageneric names and concepts follow Corner & Bas (1962) and Bas (1969).

Results

Taxonomy

Amanita coacta Bas

FIGS. 1–2

GENBANK FJ236806, FJ236807

MACROCHARACTERS — PILEUS 45–69 mm diam, plane-convex, sometimes slightly depressed at centre or concave, dark grayish brown ($N_{80}A_{30}M_{50}$ to $N_{80}A_{60}M_{60}$) at center, somewhat slightly paler toward the margin, margin rather densely sulcate-striate, dry to subviscid, with gray to brownish gray patches of the universal veil scattered or concentrated at center, occasionally lacking volval remnants on pileus. LAMELLAE free, white, crowded, with or without dark grayish edge; lamellulae scarce or absent, truncate. STIPE 62–100 × 4–8(apex)–8–11(base) mm, subcylindrical, thicker toward the base but without bulb, central, hollow, pale cream with small grayish to grayish-brown fibrils, exannulate. VOLVA at base of stipe, felted-submembranous, with whitish to grayish short fibrils, thin and fragile, easily breakable into grayish patches clearly separated, often forming incomplete transverse zones.

MICROCHARACTERS — BASIDIOSPORES [360/18/16] 8.7–10(–11.2) × (6.2–)7.5–8.7(–10) μm [$L = 8.05\text{--}10.04$; $L' = 9.46$; $W = 6.85\text{--}8.4$; $W' = 7.76$; $Q = (1.12\text{--})1.15\text{--}1.33(–1.4)$; $Q = 1.17\text{--}1.3$; $Q' = 1.22$], subglobose to broadly ellipsoid, rarely ellipsoid, inamyloid, colorless, hyaline, smooth, thin-walled, with large guttule or frequently with precipitated internal content; apiculus lateral to sublateral. BASIDIA (23–)31–46(–56) × (8.7–)10–12.5(–13.7) μm , clavate, thin-walled, frequently with precipitated internal content, 4-spored, with sterigmata up to 6.2 μm . PLEUROCYSTIDIA and CHEILOCYSTIDIA absent. SUBHYMENIUM cellular, up to 25 μm width, as 2–4 layers of more or less isodiametric to irregular cells, (8.7–)10–18.7(–21) × (6.2–)8.7–12(–15) μm ; $W_{\text{st-near}} = 15\text{--}31$, $W_{\text{st-far}} = (32\text{--})37\text{--}44$. LAMELLA TRAMA bilateral, slightly divergent, with $W_{\text{cs}} = 25\text{--}37$, composed of thin-walled hyphae, hyaline, septate, sometimes branched and slightly inflated, 3.7–8.7(–15) μm diam., and usually with divergent terminal inflated elements up to 35 μm diam. PILEUS CONTEXT undifferentiated, approximately 162 μm thick, composed of thin-walled hyphae, hyaline, septate, 3.7–8.7(–12.5) μm diam. STIPE CONTEXT longitudinally acrophysalidic, with undifferentiated hyphae 3.7–6.2 μm diam., acrophysalides thin-walled, 162–225 × 27–34 μm , and sometimes with oleiferous hyphae up to 12.2 μm diam. PILEIPELLIS as cutis up to 210 μm thick, composed of thin-walled hyphae, 2.5–10 μm diam., with brown vacuolar pigment and with few conspicuous subradial elements, with or without an ixocutis layer up to 50 μm thick on top, sometimes with distinctive volval remnants. UNIVERSAL VEIL ON PILEUS consisting of undifferentiated hyphae 2.5–6.2 μm diam., thin-walled, slightly yellowish,



Figure 1: *Amanita coacta* collections. a. F. Karstedt & Menolli Jr. 841. b. M. Capelari & D.M. Vital 4189. c. M. Capelari et al. 4269. d. M. Capelari & F.V. Neves 4309. e. Menolli Jr. et al. 99. f-g. Menolli Jr. et al. 126. h. Menolli Jr. et al. 101. i. M. Capelari & F.V. Neves 4308. j-l. M. Capelari et al. 4148. Scale bar = 1 cm.

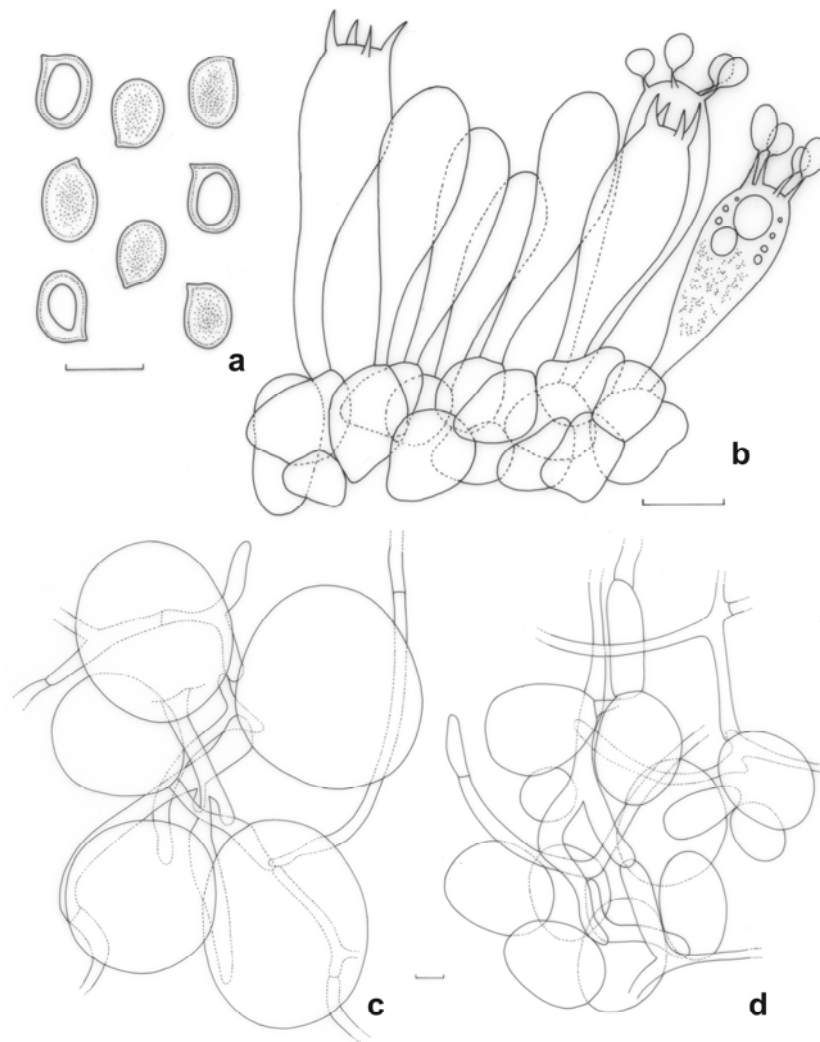


Figure 2: *Amanita coacta* (Menolli Jr. et al. 101). a. Basidiospores (guttulate or with precipitated internal content). b. Basidia and subhymenium. c. Universal veil remnants on pileus. d. Volval elements on stipe. Scale bars = 10 µm.

septate, moderately branched, loosely interwoven, with inflated terminal elements and ovoid, globose or subglobose cells, (25–)30–60(–69) × (19–)22–51(–65) µm, with light brown vacuolar content, thin-walled. UNIVERSAL VEIL ON BASAL PART OF STIPE consisting of undifferentiated hyphae, 2.5–7.5

µm diam., thin-walled, slightly yellowish, septate, moderately branched, loosely interwoven, with inflated terminal elements and ovoid, globose or subglobose cells, (20–)25–38(–50) × (16–)20–34(–44) µm with light brown vacuolar content, thin-walled. Clamps absent in all parts examined.

HABITAT AND SUBSTRATE — Solitary or in small groups (two to three basidiomata near) on soil in a remnant of Atlantic forest in São Paulo City.

SPECIMENS EXAMINED — **BRAZIL. SÃO PAULO STATE:** São Paulo, PARQUE ESTADUAL DAS FONTES DO IPIRANGA — 29.I.1960, Fidalgo & Furtado s.n. (SP); 21.I.1987, Pegler 3810 (SP); 20.I.1999, M. Capelari et al. s.n. (SP); 20.II.2002, M. Capelari et al. 4148 (SP); 19.XI.2002, M. Capelari & D.M. Vital 4189 (SP); 02.IV.2003, U.C. Peixoto s.n. (SP); 16.II.2004, M. Capelari et al. 4269 (SP); 15.II.2005, M. Capelari & F.V. Neves 4308, 4309 (SP); 03.III.2005, G.R. Leal s.n. (SP); 07.IV.2005 M. Capelari s.n. (SP); 11.I.2007, F. Karstedt & Menolli Jr. 841 (SP); 14.II.2007, Menolli Jr. et al. 99, 100, 101 (SP); 20.IV.2007, Menolli Jr. et al. 126 (SP).

COMMENTS — *Amanita coacta* is a typical neotropical species of subgenus *Amanita*, section *Vaginatae* (Fr.) Quél. due to its inamyloid basidiospores, a densely sulcate pileus margin, a subcylindrical stipe without a bulbous base, and submembranous-felted volva. However, our southeastern Brazilian collections present some differences when compared to the description in the protologue of the type from Amazonas State, Northern Brazil.

Our collections have subhymenium cells slightly shorter and cells of the universal veil on pileus longer than those described in the protologue [25(–35) µm for subhymenium cells and 20–35 µm for volval remnants cells].

The information provided here improves our knowledge of the micro-characters of *A. coacta* including range in spore size and shape and clarifies some data, such as the lamella trama which according to the protologue is “impossible to study in type” (Bas 1978). Other macro-morphological variations are assigned to *A. coacta* which were not described in the protologue, because the type is a single basidioma (collected in 1977 by Singer) which Bas had probably not examined fresh. Therefore, we report new morphological variation such as occurrence in small groups and predominantly in summer months; pileus commonly plane-convex and occasionally without volval remnants; lamella edge sometimes dark grayish; stipe with measurable differences between apex and base; and volva often forming incomplete transverse zones.

In one collection of *A. coacta*, viz. M. Capelari et al. 4148, the stipe surface, at first sight, appears to have concentric annuli one-third upward from the base (Figure 1j–l). However, this is a misinterpretation because the “annuli” are actually the result of the stipe surface cleaving, probably due to exposure to the sun in an open field instead of the shaded margin of trails in the forest where all other specimens were collected.

These morphological variations could be easily understood when there is a large collection from the same area, and one should not disregard the age of the basidiomata and environmental conditions such as recent rain, humidity and desiccation. Besides, preliminary molecular studies of DNA sequences (nLSU) from these collections (data not shown) showed high similarity between specimens from different collections that included morphological variations such as the absence or presence of patches on pileus and confirmed its position inside the section *Vaginatae*.

Amanita coacta is very similar to *A. craseoderma* Bas, also described from Amazonas State, but the latter has (sub)globose basidiospores and pigmented hyphae in the pileipellis up to 25 µm wide (Bas 1978). The third species described in the section *Amanita* for the same area is *A. crebresulcata* Bas, but it differs in having a saccate volva leaving no remnants on pileus.

Amanita crebresulcata was previously reported for Parque Estadual das Fontes do Ipiranga by Grandi et al. (1984) as "*Amanita* sp. aff. *crebresulcata*" based on a collection of Fidalgo & Furtado s.n. (SP46749). Similarly, Pegler (1997) based on same collection and an additional collection, Pegler 3810 (SP214459). The collection SP46749 is poorly preserved, without volva and base of the stipe, but has distinctive patches on pileus, the latter unmentioned for *A. crebresulcata*. Thus, this collection probably represents a specimen of *A. coacta*. A re-examination of SP214459 also demonstrated that it represents *A. coacta* and not *A. crebresulcata*. The records of *A. crebresulcata* for São Paulo State as reported by Grandi et al. (1984) and Pegler (1997) must thus be invalidated.

Another *Amanita* species may occur in this area. A single specimen was collected, viz. 07.IV.2005 M. Capelari s.n. (SP), which seems to be *A. coacta* based on micro-morphological characters, although it differs in its macroscopic appearance. This specimen when collected probably was not completely developed and so it is not possible to confirm whether it is another specimen of *A. coacta* or a second *Amanita* species.

Species of *Amanita* recorded from Brazil

In the available literature, nineteen taxa of *Amanita* are reported from Brazil. These taxa are presented in alphabetical order along with their reference of publication and distribution in Brazilian States (Table 1).

When *A. spissa* (Fr.) P. Kumm. was recorded from Brazil by Rick (1906), he also proposed two varieties, viz. *A. spissa* var. *alba* Rick [nom. illegit., non Quél.] and *A. spissa* var. *laeta* Rick. Later Rick (1930, 1937, 1961) cited *A. strobiliformis* (Paulet ex Vittad.) Bertill. and *A. bresadolae* (Rick) Rick [nom. illegit., non Schulzer] all from Rio Grande do Sul State. Singer (1953), reviewing Rick's species, renamed *A. bresadolae* as *Lepiota crassior* Singer,

but did not comment on the varieties of *A. spissa* published by Rick in 1906. Bas & Meijer (1993) considered *A. spissa* var. *laeta* as a possible synonym of *A. grillipes* Bas & de Meijer described from Paraná State. There is no subsequent mention of *A. spissa* var. *alba* in the literature, and the latter authors considered this variety as *nomina dubia*, since there is neither indication of the specimen in Rick's publication nor preserved material. *Amanita spissa* and *A. strobiliformis* are European taxa and are not included in the key because they were cited only by Rick (1906, 1930, 1937, 1961) and probably do not occur in Brazil.

Table 1: Species of *Amanita* recorded from Brazil, geographic distribution and references list.

Species	Distribution in Brazil ^a	References
<i>Amanita ameghinoi</i> (Speg.) Singer	SP	Pegler (1997)
<i>Amanita campinaranae</i> Bas ^b	AM	Bas (1978)
<i>Amanita chrysoleuca</i> Pegler	PR	Meijer (2006)
<i>Amanita coacta</i> ^b	AM, SP*	Bas (1978), Grandi et al. (1984) and Pegler (1997) as <i>A. crebresulcata</i>
<i>Amanita craseoderma</i> ^b	AM, RO	Bas (1978), Capelari & Maziero (1988)
<i>Amanita crebresulcata</i> ^b	AM, PR, PE	Bas (1978), Meijer (2006) as " <i>A. cf. crebresulcata</i> ", Wartchow & Maia (2007)
<i>Amanita grillipes</i> ^b	PR, RS	Rick (1906) as " <i>Amanita spissa</i> var. <i>laeta</i> ", Bas & Meijer (1993), Meijer (2001, 2006)
<i>Amanita lanivolva</i> Bas ^b	AM	Bas (1978)
<i>Amanita lilloi</i> Singer	PE	Wartchow et al. (2007)
<i>Amanita muscaria</i>	PR, RS, SC, SP	Homrich (1965), Fosco-Mucci & Yokomizo (1985), Figueiredo et al. (1996), Giachini et al. (2000), Meijer (2001, 2006), Giachini et al. (2004), Sobestiansky (2005)
<i>Amanita pantherina</i> var. <i>multisquamosa</i>	SC	Giachini et al. (2000, 2004)
<i>Amanita phaea</i> Bas (nom. prov.) ^b	AM	Bas (1978)
<i>Amanita rubescens</i>	RS	Sobestiansky (2005)
<i>Amanita spissa</i>	RS	Rick (1906, 1937, 1961)
<i>Amanita spissa</i> var. <i>alba</i>	RS	Rick (1906)
<i>Amanita strobiliformis</i>	RS	Rick (1930, 1937, 1961)
<i>Amanita sulcatissima</i> Bas ^b	AM	Bas (1978)
<i>Amanita xerocybe</i> Bas ^b	AM	Bas (1978)

^a = Brazilian States: AM = Amazonas, PR = Paraná, PE = Pernambuco, SP = São Paulo, SC = Santa Catarina, RS = Rio Grande do Sul, RO = Rondônia; ^b = type locality in Brazil; * = species recorded in this work

Amanitopsis plumbea Rick [nom. illegit., non (Schaeff.) J. Schröt.] is another problematic species described by Rick (1937) from Brazil. Bas (1978) studied the lectotype material (J. Rick 12.220, PACA) and considered this taxon as an insufficiently known species, since it was not possible to assign it to either *A. crebresulcata* or *A. coacta* and a third taxon may be involved.

Key to species of *Amanita* occurring in Brazil

1. Basidiospores amyloid; pileal margin usually smooth, rarely sulcate-striate; short gills often attenuate (subgenus *Lepidella*) 2
1. Basidiospores inamyloid; pileal margin radially sulcate-striate; short gills nearly always truncate (subgenus *Amanita*) 7
- 2(1). Pileal margin not appendiculate; surface often deeply colored; basidiospores globose to ellipsoid, mostly < 10 µm, rarely up to 12 µm long; annulus membranous, rarely fugacious (Section *Validae*) 3
2. Pileal margin appendiculate; surface rarely deeply colored; basidiospores globose to bacilliform, rather often > 10 µm; annulus floccose to fugacious (Section *Lepidella*) 5
- 3(2). Pileus about 6 cm wide, convex to depressed when mature, white to pallid grayish with gray volval crust at center, viscid; basidiospores globose to subglobose, 5.5–7.5 × 5.5–6.5 µm *A. campinaranae*
3. Pileus usually wider than 6 cm, conical, hemispheric-convex to plane-convex, rarely depressed 4
- 4(3). Pileus about 6.5 cm wide, conical, very dark brown with scattered small grayish warts and patches; stipe white above and gray below annulus, with scattered small volval warts; basidiospores characters unknown *A. phaea*
4. Pileus 6–12(–15), convex to applanate or finally depressed, reddish brown or more yellowish and paler, with more or less concentrically arranged whitish to grayish or grayish brown squamules; stipe whitish soon discoloring pinkish and concolor with pileus, with slight volval granulation; basidiospores ellipsoid, 7.5–10 × 4.5–5.5 µm *A. rubescens*
- 5(2). Pileus hemispherical or conic-convex to plane-convex, uniformly dark brown to somewhat paler grayish brown *A. grallipes*
5. Pileus subglobose or hemispheric to plane-convex white to pale pinkish orange or light beige with patches 6
- 6(5). Pileus narrower than 4 cm, usually about 2–4 cm; basidiospores subglobose to broadly ellipsoid, mostly < 10 µm, about 7.5–9.5 × 6.5–7.5 µm, basidia 4-spored *A. lilloi*
6. Pileus usually wider than 4 cm; basidiospores ellipsoid, rather often > 10 µm, about 11–13 × 8–10 µm, basidia mostly 2-spored *A. ameghinoi*
- 7(1). Stipe with a bulbous base; volva usually friable, sometimes limbate; annulus absent or present (Section *Amanita*) 8
7. Stipe without basal bulb; volva saccate to sub-membranous-felted, more rarely friable; annulus absent (Section *Vaginatae*) 13
- 8(7). Pileus often red, orange or yellow covered with white or yellowish patches or flocculose-pulverulent velar remnants 9
8. Pileus brown, ochraceous brown, brownish, whitish to grayish, covered with gray, white to grayish or brownish ochraceous warts and patches 10
- 9(8). Pileus 8–18(–25) cm wide, light orange to deeply red, with white patches; basidiospores ellipsoid, 9–11.5 × 6–8 µm *A. muscaria*

9. Pileus 2.5–3.5 cm wide, dry, deep chrome yellow to orange-yellow, covered with yellowish ochraceous, flocculose-pulverulent velar remnants; basidiospores subglobose to ellipsoid, $7\text{--}9.5 \times 4.5\text{--}6 \mu\text{m}$ *A. chrysoleuca*
- 10(8). Clamps absent; pileus 4–6.7 cm wide, sordid whitish to ochraceous with brownish ochraceous center; basidiospores globose to subglobose, $8\text{--}9 \times 7.5\text{--}9 \mu\text{m}$ *A. xerocybe*
10. Clamps present; pileus about 4 cm wide 11
- 11(10). Annulus fragmentary *A. pantherina* var. *multisquamosa*
11. Annulus always absent 12
- 12(11). Volva saccate, membranous, gray-brown, enclosing one-quarter to one-third of stipe like a sock *A. lanivolva*
12. Volval remnants at base of stipe arising from upper part of bulb, appressed, pale brownish-gray, with subtomentose-sublanose surface, at one side of stipe forming a thin submembranous limb above bulb *A. sulcatissima*
- 13(7). Volva friable, forming a dark gray-brown sub-floccose belt at base of stipe and evanescent small dark brown warts on cap *A. craseoderma*
13. Volva saccate or submembranous-felted, white to pale buff or grayish, felted to, at most, appressedly fibrillose on outside limb 14
- 14(13). Volva membranous, narrowly saccate, white, leaving no remnants on pileus; basidiospores subglobose to broadly ellipsoid, rarely ellipsoid, $(8\text{--})8.5\text{--}10\text{--}(11) \times (6.5\text{--})7\text{--}8.5\text{--}(9) \mu\text{m}$ *A. crebresulcata*
14. Volva felted sub-membranous, tending to break up into small appressed flat gray patches at stipe base and around cap center; basidiospores subglobose to broadly ellipsoid, $8.7\text{--}10\text{--}(11.2) \times (6.2\text{--})7.5\text{--}8.7\text{--}(10) \mu\text{m}$ *A. coacta*

According to the classification of Corner & Bas (1962), the Brazilian species are distributed in the two subgenera *Amanita* and *Lepidella* and in four sections *Amanita*, *Vaginatae*, *Validae* and *Lepidella*. But, the infrageneric classification of *A. lanivolva* should be regarded with caution. This species was firstly described in section *Vaginatae* (Bas 1978) by the possession of saccate volva. Nevertheless, the presence of a small, but distinct basal bulb was used by Simmons et al. (2002), from collections of Guyana, to classify it in section *Amanita*, although in this latter section, the presence of saccate volva is uncommon

Of the new species described from Bas (1978), only *A. craseoderma* (Capelari & Maziero 1988) and *A. crebresulcata* (Meijer 2006, Wartchow & Maia 2007) were further found and mentioned outside Amazonas State. Simmons et al. (2002) also recorded *A. lanivolva* and *A. xerocybe* from Guyana.

According to Bas (1978), a provisional name was given for *A. phaea*, because there were no basidiospores in the type material, but other characters such as smooth and non-appendiculate margin of the pileus, friable volva and attenuate lamellulae, are enough for its classification in the section *Validae*.

The results of this paper strongly support the necessity of collecting and studying *Amanita* in South America because very little is known about its neotropical species.

Acknowledgments

The authors thank Dr. Clark L. Ovrebo, University of Central Oklahoma, and Dr. Zhu L. Yang, Kunning Institute of Botany, for critical review of the manuscript; Dr. Rodham E. Tulloss, New Jersey (USA), for his advice in this study; Dr. Maria Helena Pelegrinelli Fungaro, Universidade Estadual de Londrina, for DNA sequencing; Fernanda Karstedt, Instituto de Botânica, for taking photographs of some specimens; Anderson Luis dos Santos, Instituto de Botânica, for assistance with formatting the plates; Klei R. Sousa for preparing the illustrations; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the support and grant to the first author; and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 04/04319-2) for financial support.

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CAPÍTULO II

New record and two new species of *Pluteus* (*Pluteaceae*, *Agaricales*) from Brazil*

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*Artigo a ser submetido para publicação na revista *Mycologia*.

New record and two new species of *Pluteus* (*Pluteaceae*, *Agaricales*) from Brazil

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Abstract: Two new species of *Pluteus*, *P. concavus* and *P. bulbomarginatus*, are described and illustrated from specimens collected in two remnants of Atlantic forest in São Paulo City, SP, Brazil. *Pluteus concavus* is characterized by a densely fibrillose and deeply concave pileus, whereas *P. bulbomarginatus* has a stipe with a grayish brown subbulbous-marginate base. *P. concavus* belongs to section *Hispidoderma* and *P. bulbomarginatus* to section *Pluteus*. *Pluteus longistriatus* and *P. umbrinoalbidus*, collected in the same area, are also described, illustrated and recorded. *Pluteus longistriatus* represents a new record for Brazil.

Key words: *Basidiomycota*, Atlantic rainforest, diversity, taxonomy

INTRODUCTION

The genus *Pluteus* Fr. is characterized by free lamellae, pinkish spore print, absence of both annulus and volva, basidiospores inamyloid, cystidia often metuloid and hymenophoral trama inverse (Singer 1986). The currently accepted infrageneric classification of the genus *Pluteus* subdivides it into three sections (*Pluteus* Fr., *Hispidoderma* Fayod and *Celluloderma* Fayod) based on morphological characters such as the structure of pileipellis and characteristics of pleurocystidia (Singer 1958, 1986).

Knowledge about this genus in Brazil includes 20 species and varieties with Brazilian types described by Hennings (1900, 1904a, 1904b), Bresadola (1920), Rick (1930, 1938, 1961) and Singer (1953, 1956, 1958, 1989). However, many of them represent non-preserved specimens, misidentifications, *nomina dubia*, synonyms or species of other genera, as considered by Singer (1953, 1958) for the Brazilian species described by Hennings (1900, 1904a, 1904b), Bresadola (1920) and Rick (1930, 1938, 1961).

Other contributions regarding *Pluteus* in Brazil include those made by Grandi *et al.* (1984), Raithelhuber (1991), Stijve & Meijer (1993), Pegler (1997), Meijer (2001, 2006), Wartchow *et al.* (2004, 2006), and Drechsler-Santos *et al.* (2007). However, most of these papers are lists without description, except the publications by Wartchow *et al.* (2004, 2006), which include a complete description of six species for Rio Grande do Sul State, Brazil.

In this article, four species of *Pluteus* are reported based on specimens recently collected at the Parque Estadual da Cantareira and Parque Estadual das Fontes do Ipiranga, both remnants of the Atlantic forest in São Paulo City, SP, Brazil. Two of them are described as new, one represents the first record for Brazil, and the other is a Brazilian species known only by the type material.

MATERIAL AND METHODS

The specimens were collected at the Parque Estadual da Cantareira and Parque Estadual das Fontes do Ipiranga, both remnants of the Atlantic forest in São Paulo City, SP, Brazil and deposited at herbarium SP. The macroscopical descriptions and illustrations of basidiomata were based on fresh material. Color terms are according to Küppers (1979). For microscopic analyses, the dried material was rehydrated in 70% ethanol, followed by 5% KOH or Melzer's reagent. All microscopic illustrations were made with the aid of a drawing tube. The notation "[*a/b/c*]" at the beginning of a set of the basidiospores data is to be read "*a* spores were measured from *b* basidiomata taken from *c* collections". Q represents the range of

length/width quotient for all spores measured. The basidiospores were measured in lateral view and the terms denoting its shape are according to Bas (1969). For the pileipellis cells, the measure of pedicel is given with the spheropedunculate cells and also separated. Generic and infrageneric concepts follow Singer (1986).

TAXONOMY

Pluteus bulbomarginatus Menolli & Capelari, sp. nov.

FIGS. 1A–B, 2

Similis *Pluteus stephanobasis* Singer striis persistentibus atrobrunneis vel atrofuscis ad basim stipitis, sed habitu minori (35 mm diam.), pleurocystidiis metuloideis solum *cervinus* typi et basidiosporis uniformibus, maioribus [(6.2–)7.5–8.7 × (5–)6.2–7.5 μm] differt.

Pileus 35 mm diam., plane, slightly depressed around the low umbo, brown to dark hazel (N₇₀A₈₀M₇₀), finely pruinose and darker at center, slightly discoloring with paler buff shades towards the margin, margin slightly sulcate-striate. *Lamellae* free, white then pinkish, crowded, with lamellulae. *Stipe* 40 × 2(apex)–3(base) mm, slightly tapering towards the apex, with a subbulbous-marginate base, central, cream with grayish brown base, slightly longitudinally striate especially at base, with slightly strigose basal mycelium.

Basidiospores [20/1/1] (6.2–)7.5–8.7 × (5–)6.2–7.5 μm (Q = 1.16–1.25) broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* 21–29(–31) × (7.5–)8.7–10 (–11.2) μm, versiform, thin-walled, 4-spored, sometimes with small scattered guttulae. *Pleurocystidia* (52–)57–68(–71) × 12.5–18.7 μm, fusoid-ventricose, metuloids of *Cervinus*-type, abundant, hyaline, sometimes with a hyaline amorphous substance, rather strongly thick-walled at apex (up to 6.2 μm wide) and collapsing towards the base, apices usually with 4 lateral prongs or occasionally up to 8 suberect, acute or obtuse horns (up to 6.2 μm long). *Cheilocystidia* (26–)34–44(–46) × (8.7–)10–13.7 μm, clavate-vesiculose, very abundant, usually with a moderately long pedicel, hyaline, thin-walled. *Lamella edge* sterile. *Lamella trama* bilateral convergent, up to 50 μm width, composed by thin or slightly thick-walled hyphae, 1.2–5 μm diam., hyaline. *Pileus context* undifferentiated, approximately 125 μm thick, composed by thin or slightly thick-walled hyphae, usually up to 13.7 μm diam. or sometimes inflated up to 31 μm diam., hyaline. *Pileipellis* a repent epicutis up to 150 μm thick, composed by slightly thick-walled hyphae, 6.2–10 μm diam., sometimes with the subterminal elements slightly inflated up to 15 μm diam., elongated, septate, with rounded apex or with an acute apex and narrowing end cell (3.7–6.2 μm diam.), with dissolved brown vacuolar content. *Clamp connections* absent in all parts examined.

Habit and habitat. Solitary, on decaying wood.



FIG. 1. A–B. *Pluteus bulbomarginatus* (holotype). C–D. *P. concavus* (holotype). E. *P. longistriatus* (L.A.S. Ramos & F. Karstedt 25). F–G. *P. umbrinoalbidus* (Menolli Jr. et al. 116). Scale bars = 1 cm.

Etymology. The name refers to the characteristic morphology of the stipe base.

Specimens examined. BRAZIL. SÃO PAULO: São Paulo. Parque Estadual da Cantareira, Núcleo Engordador, 24-IV-2007, Menolli Jr. et al. 131 (HOLOTYPE SP).

Comments. This species is characterized by the plane pileus, brown to dark hazel, with a low umbo and slightly depressed around the center, margin slightly sulcate to sulcate-striate, stipe with a subbulbous-marginate base and a slightly strigose basal mycelium, pleurocystidia of the *Cervinus*-type, with a strongly thick-walled and up to 8 suberect, acute or obtuse horns.

Pluteus bulbomarginatus is closely related to *P. stephanobasis* Singer from Argentina. Singer (1958) described the stipe base of *P. stephanobasis* with a “constant and persistent deep brown to deep fuscous fibrillose line” which must be compared to the stipe base of *P. bulbomarginatus*. However, *P. bulbomarginatus* is quite different from *P. stephanobasis*, because the latter has a more robust habit, dimorphic basidiospores, usually shorter [$4.8\text{--}6.2 \times 3.3\text{--}4.2\text{--}(4.8) \mu\text{m}$], or in small number a second type of macrobasidiospores ($7.5\text{--}9 \times 5.5\text{--}6 \mu\text{m}$), pleurocystidia metuloids of two types, of the *Magnus*- and *Cervinus*-type (Singer, 1958).

Pluteus bulbomarginatus belongs to section *Pluteus* due to pileipellis with elongate hyphous elements and the presence of metuloids.

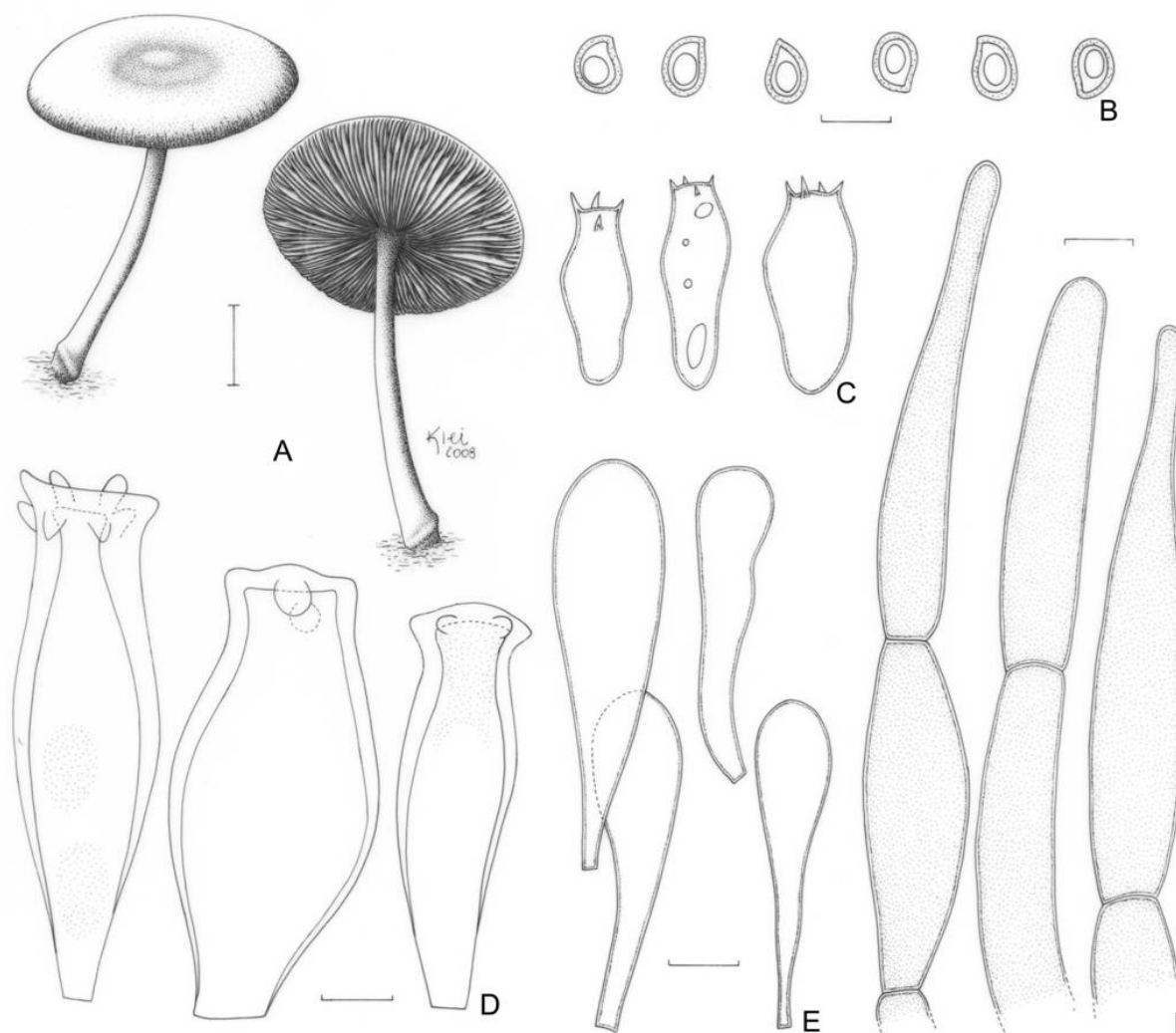


FIG. 2. *Pluteus bulbomarginatus* (holotype). A. Basidioma. B. Basidiospores. C. Basidia. D. Pleurocystidia. E. Cheilocystidia. F. Pileipellis elements. Scale bars: A = 1 cm, B–F = 10 µm.

***Pluteus concavus* Menolli & Capelari, sp. nov.**

FIGS. 1C–D, 3

Similis *Pluteus albstipitatus* (Dennis) Singer superficie pilei perdense radialiter fibrillosa et velutina ad fibrilloso-punctam in centro sed pileo profunde concavo et absque margine sulcata nec striata, et basidiosporis globosis aut leviter ellipsoideis, uniformibus differt.

Pileus 1.1–1.5 mm diam., deeply concave, not umbonate, dark brown to slightly grayish (N₈₀A₃₀M₄₀), surface densely fibrillose with granular punctations at center, composed of dark and light brown fibrils radially arranged forming a mixture of brown shades, pileus flesh becoming visible and translucently pinkish between the fibrils at the marginal zone due to lamellae color, margin neither sulcate nor striate. *Lamellae* free to remote, pinkish, moderately crowded, with lamellulae. *Stipe* 20–35 × 2(apex)–3(base) mm, slightly tapering

towards the apex, central, white-cream with grayish base, slightly longitudinally striate, with scanty mycelium at base.

Basidiospores [40/2/1] (6.2–)7.5–8.7 × 6.2–7.5(–8.7) μm (Q = 1–1.2), globose, occasionally broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, sometimes guttulate. *Basidia* (21–)23–30 × (7.5–)8.7–10(–11.2) μm, versiform to clavate, thin-walled, 4-spored, sometimes with small scattered guttulae. *Pleurocystidia* 50–67(–81) × (10–)12.5–18.7(–22) μm, slightly ventricose to lageniform, rounded apex or sometimes with an apical constriction, hyaline, very numerous, thin to slightly thick-walled. *Cheilocystidia* 40–55 × 12.5–18.7 μm, like pleurocystidia, but very rare. *Lamella edge* apparently sterile or subheteromorphous. *Lamella trama* bilateral convergent, up to 50 μm width, composed by thin or thick-walled hyphae, 3.7–11.2 μm diam., hyaline. *Pileus context* undifferentiated, approximately 37 μm thick, composed by thick-walled hyphae, slightly inflated, 3.7–11.2 μm diam., hyaline, septate. *Pileipellis* a repent cutis, up to 112 μm thick, with few ascendent and conspicuous subradial elements, composed by thin-walled hyphae, 7.5–11.2 μm diam., elongated, septate, with rounded to subacute apex (up to 5 μm diam.) and dissolved brown vacuolar content. *Clamp connections* absent in all parts examined.

Habit and habitat. Subgregarious (two basidiomata near), on wood.

Etymology. The name refers to the pileus shape.

Specimens examined. BRAZIL. SÃO PAULO: São Paulo. Parque Estadual da Cantareira, Núcleo Engordador, 24-IV-2007, *Menolli Jr. et al.* 129 (HOLOTYPE SP).

Comments. *Pluteus concavus* is characterized by the deeply concave pileus, densely fibrillose with dark and light brown fibrils radially arranged and granular punctations at center, pleuro- and cheilocystidia similar in shape, and basidiospores usually globose.

This species is closely related to *P. albstipitatus* (Dennis) Singer described from Trinidad, especially due to pileus surface very densely radially fibrillose and velutinous to fibrillose-punctate in the center (Singer 1958). However, *P. albstipitatus* is different from *P. concavus* in pileus shape, which is usually described as conic-campanulate to convex, with a broad and low umbo, and a sulcate-striate margin (Singer 1958, Pegler 1983, Wartchow *et al.* 2006). Besides, Singer (1958) observed a wide variation in basidiospores size (5.8–10.3 × 4.7–9 μm), which were classified into three classes (small, normal, and gigantic) and never as globose. *Pluteus concavus* belongs to section *Hispidoderma* due to pileipellis with elongate hyphous elements and absence of metuloids.

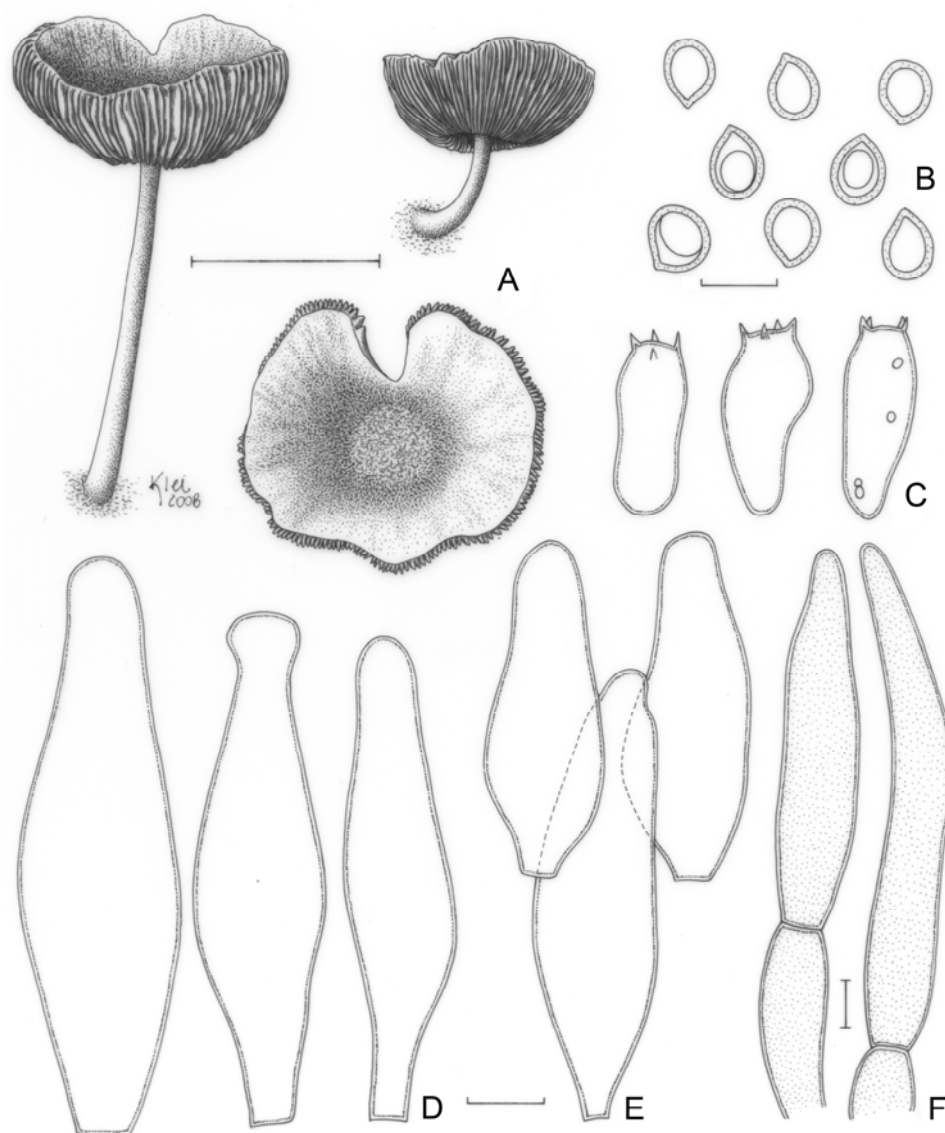


FIG. 3. *Pluteus concavus* (holotype). A. Basidioma. B. Basidiospores. C. Basidia. D. Pleurocystidia. E. Cheilocystidia. F. Pileipellis elements. Scale bars: A = 1 cm, B–F = 10 µm.

Pluteus longistriatus (Peck) Sacc., *Sylloge Fungorum* 5: 670. 1887.

FIGS. 1E, 4

Pileus 25–42 mm diam., conic-convex or campanulate to plane-convex, slightly umbonate and sometimes slightly depressed around the umbo, completely dark brown when young, then pale fuscous to light brown-gray ($A_{80}M_{60}C_{60}$), center distinctly fibrillose, sometimes forming granules or flocons, dark brown fibrils more concentrated at the center and more spaced and discoloring radially towards the margin, margin deeply sulcate-striate over at least one half of the radius. *Lamellae* free, pinkish, with lamellulae. *Stipe* 43–52 × 1–3(apex)–2–4(base) mm, central, hollow, slightly tapering towards the apex, white-cream, sometimes with scanty brownish fibrils at the base and white basal mycelium.

Basidiospores [60/3/3] 6.2–7.5(–8.7) × 5–6.2 µm ($Q = 1.2–1.5$), broadly ellipsoid, occasionally ellipsoid, inamyloid, hyaline, smooth, thick-walled, sometimes guttulate. *Basidia*

(18.7–)21–24 × (7.5–)10–11.2 μm, versiform, thin-walled, 4-spored. *Pleurocystidia* (45–)50–71(–81) × (8.7–)10–24(–29) μm, slightly ventricose to lanceolate, rarely clavate, subhyaline with pale straw dissolved content, numerous, thin-walled. *Cheilocystidia* (25–)29–55(–61) × 10–18(–21) μm, like pleurocystidia, but hyaline, moderately numerous, and sometimes smaller or versiform-clavate to spheropedunculate. *Lamella edge* sterile. *Lamella trama* bilateral convergent, up to 40 μm width, composed by thin or thick-walled hyphae, 2.5–6.2 μm diam., hyaline. *Pileus context* undifferentiated, approximately 69 μm thick, composed predominately by oleiferous hyphae up to 6.2 μm diam., moderately branched, thin-walled, few septa seen. *Pileipellis* cellular up to 90 μm thick, composed by one layer of vesiculose, clavate or slightly fusoid cells, (62–)67–108(–117) × (25–)30–43(–52) μm, intermixed by elongate-fusiform or elongate-clavate dermatocystidia, (105–)135–150(–170) × (18–)22–33 (–40) μm, both types thin-walled and with dissolved or sometimes condensed brown vacuolar content. *Caulocystidia* (42–)70–120(–134) × 20–34 μm, clavate, rarely with narrow papillate apex, thin-walled, hyaline, numerous. *Clamp connections* absent in all parts examined.

Habit and habitat. Solitary or in small groups, on decaying wood.

Specimens examined. BRAZIL. SÃO PAULO: São Paulo. Parque Estadual das Fontes do Ipiranga, 20-VI-2006, *F. Karstedt et al.* 680, 681 (SP); 31-VIII-2008, *L.A.S. Ramos & F. Karstedt* 25 (SP).

Comments. *Pluteus longistriatus* was described from North America and also recorded for Argentina by Singer (1958). Singer (1956, 1958) described the stipe of *P. longistriatus* as “sometimes fuliginous-fibrillose-punctate” at the base, but did not mention either the presence or absence of caulocystidia, as observed in the Brazilian material. In the study of the type and one additional collection from Argentina, Singer (1956, 1958) described three types of pileipellis elements: sphaerocytes, elongated dermatocystidia and a transitional form. In the Brazilian material, however, only two types of pileipellis elements were observed, the elongated dermatocystidia and the clavate-vesiculose cells, which probably represent the transitional form specified by Singer (1956, 1958). Besides, Singer (1956, 1958) described the cystidia as vesiculose to ventricose, and the cheilocystidia as very similar to the pleurocystidia or often somewhat smaller and more numerous, but did not describe each type of cystidium separately. According to the drawings presented by Singer (1956) and based on the North American specimens, the pleuro- and cheilocystidia are represented like clavate-vesiculose. Nevertheless, the cystidia, also without distinction in pleuro- or cheilocystidia, as illustrated by Singer (1958) and based on the Argentinean species is obviously ventricose.

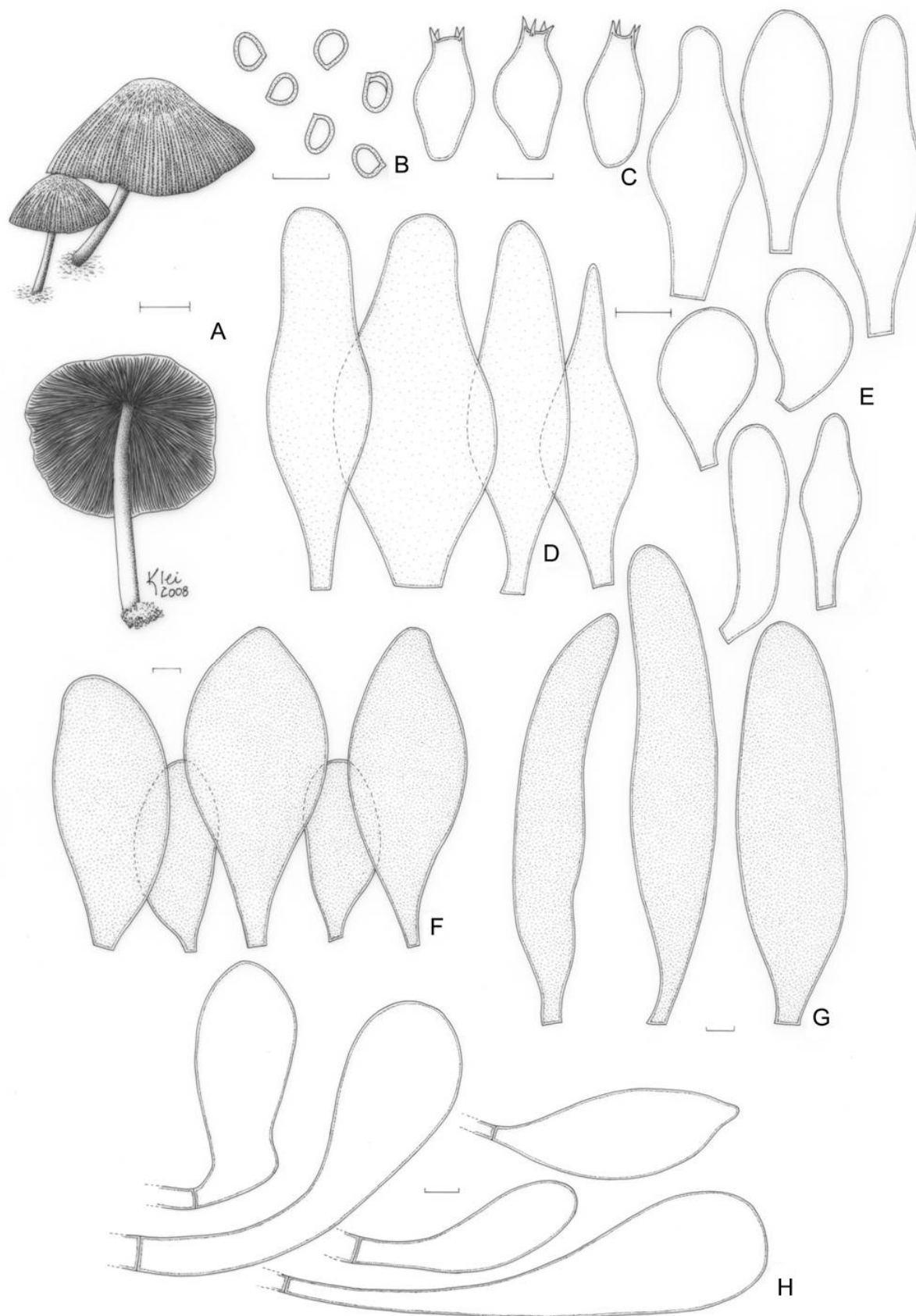


FIG. 4. *Pluteus longistriatus*. A. Basidioma (L.A.S. Ramos & F. Karstedt 25). B–H. F. Karstedt et al. 680. B. Basidiospores. C. Basidia. D. Pleurocystidia. E. Cheilocystidia. F. Pileipellis elements. G. Dermatocystidia. H. Pileocystidia. Scale bars: A = 1 cm, B–H = 10 µm.

Our material has pleurocystidia slightly ventricose like those illustrated by Singer (1958) from the Argentinean material, although the cheilocystidia show mostly varied shapes, from clavate, as illustrated from the North American specimens (Singer 1956), to spheropedunculate or slightly ventricose. Although Singer (1956, 1958) had described pleurocystidia as similar to cheilocystidia, the variations observed in his illustration probably represent the same variations in shape observed in the Brazilian collection. It is necessary more collections and other studies including molecular data from North and South American species related to *P. longistriatus* to confirm whether these morphological variations constitute an intraspecific variation, or represent an independent species. *Pluteus longistriatus* belongs to section *Celluloderma* subsection *Mixtini* due to pileipellis composed by dimorphic elements.

Pluteus umbrinoalbidus Singer, Lilloa 26: 134. 1953.

FIGS. 1F–G, 5

Pileus 17 mm diam., convex, slightly umbonate, brown-buff (A₆₀M₇₀C₆₀) to slightly reddish, finely pruinose all over, sometimes finely cracking and showing the white flesh, margin slightly appendiculate and finely eroded but apparently neither sulcate nor radially rimose. *Lamellae* free, white then pinkish, with lamellulae. *Stipe* 23 × 1 mm, equal, central, pale cream to translucent white, slightly grayish, with fine white punctuations.

Basidiospores [20/1/1] (5–)6.2(–7.5) × 5(–6.2) μm (Q = 1–1.25) broadly ellipsoid, occasionally globose, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* (18.7–)20–24 × (6.2–)7.5–8.7 μm, clavate to versiform, thin-walled, 4-spored. *Pleurocystidia* 37–59 × 7.5–20 μm, slightly ventricose to lanceolate or fusiform-clavate, hyaline, rare, sparse, thin-walled. *Cheilocystidia* (31–)35–56(–62) × (6.2–)8.7–13.7 μm, clavate to versiform-clavate, hyaline, numerous, thin-walled. *Lamella edge* heteromorphous. *Lamella trama* bilateral convergent, up to 37 μm width, composed by thin-walled hyphae, 3.7–8.7 μm diam., hyaline, septate. *Pileus context* undifferentiated, approximately 75 μm thick, composed by thin or slightly thick-walled hyphae, hyaline, septate, 3.7–7.5 μm diam. *Pilleipellis* cellular up to 75 μm thick, composed by one layer of spheropedunculate cells, (37–)44–59 × (25–)30–35 μm, with a short to medium pedicel (3.7–12.5 μm long), intermixed by lanceolate, fusoid to slightly ventricose or rarely clavate dermatocystidia, 86–128(–138) × (14–)20–32 μm, both types thin-walled and with dark brown vacuolar content, more condensed in the spheropedunculate cells than in the dermatocystidia. *Caulocystidia* not seen. *Clamp connections* absent in all parts examined.

Habit and habitat. Solitary, on wood.

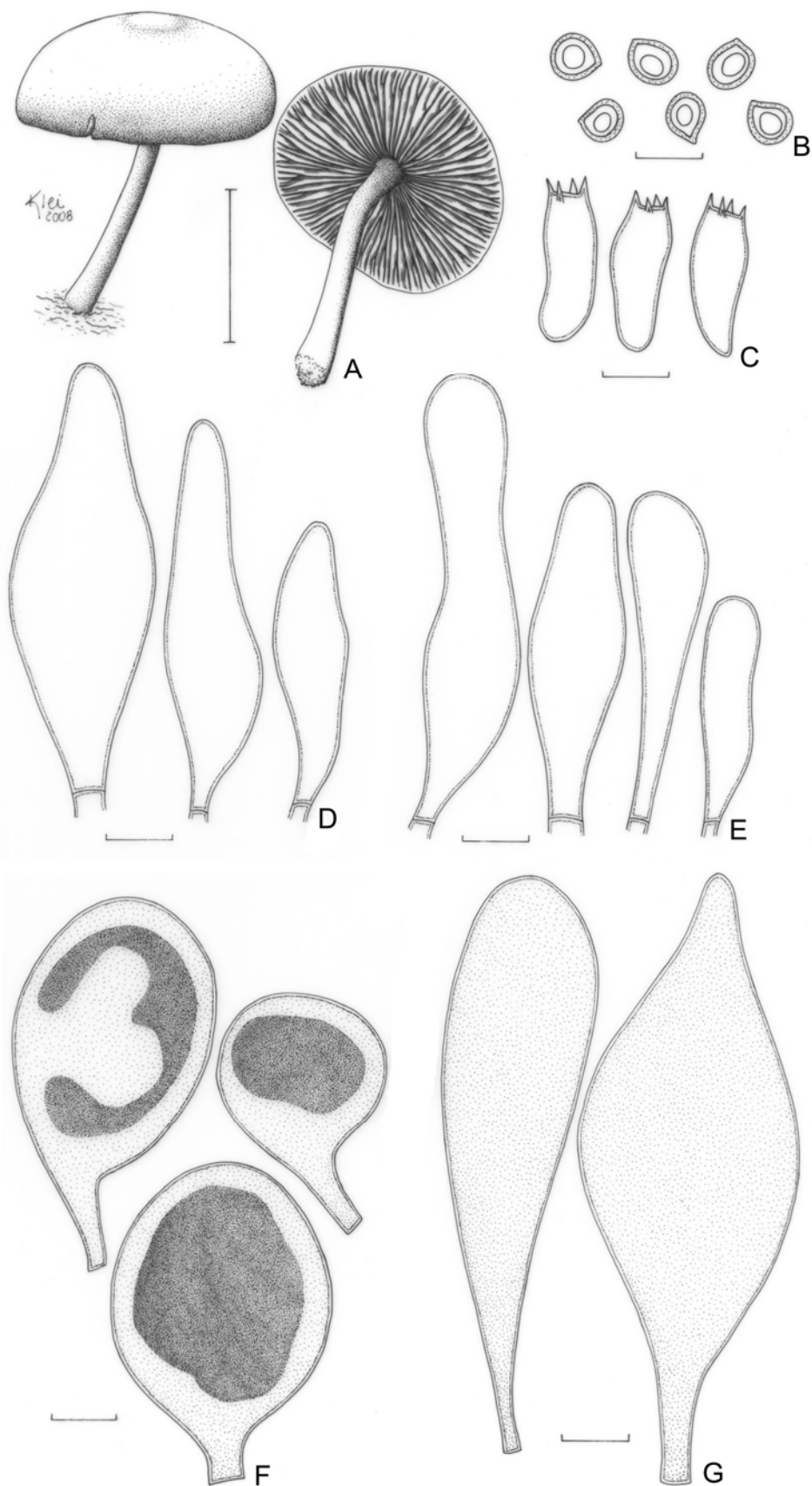


FIG. 5. *Pluteus umbrinoalbidus* (Menolli Jr. et al. 116). A. Basidioma. B. Basidiospores. C. Basidia. D. Pleurocystidia. E. Cheilocystidia. F. Pileipellis elements. G. Dermatocystidia. Scale bars: A = 1 cm, B–G = 10 μ m.

Specimens examined. BRAZIL. SÃO PAULO: São Paulo. Parque Estadual da Cantareira, Núcleo Engordador, 2-III-2007, *Menolli Jr. et al.* 116.

Comments. *Pluteus umbrinoalbidus* was described from Rio Grande do Sul State, South Brazil, and is known only by the type material (Singer 1953). Meijer (2006) recorded in a checklist the occurrence of a related species named as *P. cf. umbrinoalbidus* for Paraná State. The original description of *P. umbrinoalbidus* (Singer 1953) was based on two collections of Singer (*Singer B 110* and *Singer B 124*), but a careful reexamination done by Singer (1958) indicated that the collection *Singer B 110* represents another species, and was later described as *P. fallax* Singer. *Pluteus umbrinoalbidus* is related to other South American species such as *P. fallax*, *P. tucumanus* Singer and *P. substigmaticus* Singer. According to Singer (1958), however, *P. fallax* and *P. tucumanus* have no dermatocystidia; and, *P. substigmaticus* has a stipe macroscopically sordid because of the presence of fine fibrils forming a faint blackish punctuation on the white ground. Our material has a pileus color slightly different from that described by Singer (1958), as he attributed a deep grayish fuliginous “biskra”. Other related species like *P. seticeps*, however, vary from dark reddish brown to “biskra” (Homola 1972) and it is not possible to disregard the age of the basidiomata and environmental conditions such as recent rain, humidity and desiccation, all of which can contribute to the wide variation of color in *Pluteus* especially in the brown shades. This is the first record of *P. umbrinoalbidus* outside the type locality and the second collection since its description. It belongs to section *Celluloderma* subsection *Mixtini* due to pileipellis composed by spheropedunculate cells intermixed by dermatocystidia.

ACKNOWLEDGMENTS

The authors thank Dr. Andrew M. Minnis, Systematic Mycology & Microbiology Laboratory (USDA–ARS), for his advices in this study; Dr. Tarciso S. Filgueiras, Reserva Biológica do IBGE, and José Francisco Kuhar, FCEN Universidad de Buenos Aires, who kindly revised the Latin diagnoses; Fernanda Karstedt, Instituto de Botânica, for taking photographs and collecting some specimens; Klei R. Sousa for preparing the ink drawings; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the support and grant to the first author; and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 04/04319-2) for financial support.

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CAPÍTULO III

Records and new species of *Pluteus* from Brazil: taxonomy and molecular phylogeny*

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*Artigo a ser submetido para publicação na revista *Mycological Research*.

Records and new species of *Pluteus* from Brazil: taxonomy and molecular phylogeny

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ABSTRACT

Ten species of *Pluteus* are described and illustrated from specimens recently collected at three remnants of the Atlantic forest in São Paulo State, Southeast Brazil. Three new taxa are proposed: *P. aureovenatus*, *P. capillicomptus*, and *P. dominicanus* var. *hyalinus*, and a new status, *P. sublaevigatus* to *P. chrysophlebius* subsp. *sublaevigatus*. The occurrence of *P. fuligineovenosus*, *P. jamaicensis*, and *P. riberaltensis* var. *conquistensis* represent the first records for Brazil. In addition, a molecular study was performed based on parsimony analyses of nLSU and ITS + 5.8S sequences of Brazilian and other *Pluteus* species. The phylogenetic trees generated revealed a monophyletic clade with all *Pluteus* species divided into two clades: one with the species of section *Pluteus*, and another including members of both sections *Hispidoderma* and *Celluloderma*.

Keywords: Agaricales, Basidiomycota, South America, *Pluteaceae*

Introduction

Pluteus Fr. is a large and widely distributed genus of presumably saprophytic and non-ectomycorrhizal species (Singer 1986; Banerjee & Sundberg 1995). The genus is characterised by free lamellae, pinkish spore print, absence of both annulus and volva, basidiospores inamyloid, cystidia often metuloid, and hymenophoral trama inverse (Singer 1986).

The currently accepted infrageneric classification of the genus *Pluteus* subdivides it into three sections (*Pluteus* Fr., *Hispidoderma* Fayod and *Celluloderma* Fayod) based on morphological characteristics such as the structure of the pileipellis and characteristics of the pleurocystidia (Singer 1958, 1986). A hymeniform layer of spheropedunculate cells characterise the pileipellis of the section *Celluloderma*, while the other two sections exhibit pileipellis with elongated, pilose, or hyphous elements and differ among themselves in the presence of metuloids in section *Pluteus* or thin-walled pleurocystidia in section *Hispidoderma* (Singer 1958, 1986).

Molecular studies involving the genus *Pluteus* are restricted to nLSU gene (rDNA), without inclusion of tropical species. Moncalvo *et al.* (2002) conducted a broad study of *Agaricales*, where the *Pluteus* clade was superficially discussed. Later, Minnis *et al.* (2006) added a *Chamaeota* sequence to the analysis done by Moncalvo *et al.* (2002) and proposed a new combination in *Pluteus*.

Hennings (1900) was the first author to describe species of *Pluteus* from Brazil, with *P. scruposus* Henn. from Mato Grosso State. Later, Hennings (1904a, b) described *P. cervinus* var. *griseoviridis* Henn. from São Paulo State and *P. termitum* Henn. from Amazonas. These early species described from Brazil were not preserved and may not be *Pluteus* as mentioned by Saccardo & Trotter (1912), who recognised *P. termitum* as a possible synonym of *Collybia eurhiza* (Berk.) Höhn., and by Singer (1958), who established *P. scruposus* as a synonym of *Oudemansiella canarii* (Jungh.) Höhn. and *P. termitum* as a probably species of *Lepiota*.

Rick (1907, 1919, 1930, 1938, 1961) also described and recorded some problematic species. In total, 21 *Pluteus* taxa from Brazil (Rio Grande do Sul State), including 6 species (*P. cristatulus* Rick, *P. fibrillosus* Rick, *P. leptonia* Rick, *P. sensitivus* Rick, *P. straminellus* Rick, and *P. velatus* Rick) and 2 varieties (*P. exiguus* var. *venosus* Rick and *P. nanus* var. *podospileus* (Sacc. & Cub.) Rick) with Brazilian types. However, Singer (1953, 1958) considered several of Rick's collections as misidentifications, *nomina dubia*, synonyms, or species of other genera.

Other nine *Pluteus* species with Brazilian types were described by Bresadola (1920) and Singer (1953, 1956, 1958, 1989): *P. amazonicus* Singer, *P. cervinus* var. *brasiliensis* Bres., *P. fluminensis* Singer, *P. hylaeicola* Singer, *P. melanopotamicus* Singer, *P. riograndensis* Singer, *P. subfibrillosus* Singer, *P. umbrinoalbidus* Singer, and *P. varzeicola* Singer.

Grandi *et al.* (1984), Raithelhuber (1991), Stijve & Meijer (1993), Pegler (1997), Meijer (2001, 2006), and Drechsler-Santos *et al.* (2007) also contributed to increasing the knowledge about *Pluteus* in Brazil. However, these works are simply lists and do not include specific descriptions of the species.

Recently, Wartchow *et al.* (2004, 2006) recorded and provided a complete description of *P. albospitatus* (Dennis) Singer, *P. aquosus* Singer, *P. beniensis* Singer, *P. globiger* Singer, *P. nigrolineatus* Murrill, and *P. thomsonii* (Berk. & Broome) Dennis for Rio Grande do Sul State, Brazil. However, Rodríguez *et al.* (2008) considered that the European species *P. thomsonii* recorded by Wartchow *et al.* (2004) is probably *P. neotropicalis* Rodr.-Alcánt., which was recently described (Rodríguez *et al.* 2008) based on Mexican material previously misidentified as *P. thomsonii* (Rodríguez & Guzmán-Dávalos 1999).

The knowledge of *Pluteus* in Brazil is very confusing and incomplete due to poor descriptions, synonyms, misidentifications, lack of material preserved, and lists lacking descriptions. So far, 66 epithets were recorded for Brazil, distributed over 9 States (Amazonas, Bahia, Mato Grosso, Pará, Paraná, Rio de Janeiro, Rio Grande do Sul, Rondônia, and São Paulo), with 20 of them represented by Brazilian types. However, only 19 are certainly known or at least cited with a complete description. The remainder represents misidentifications, synonyms, species of other genera, unpreserved specimens, or are presented in lists.

Here, we report, describe and illustrate ten species of *Pluteus* recently collected in the remnants of the Atlantic forest in Southeast Brazil. We proposed three new taxa, and three new records for Brazil. Moreover, for the first time, molecular analyses with sequences of South American species based on nLSU and ITS + 5.8S data is presented.

Material and methods

Sampling

The studied materials were collected at Parque Estadual da Cantareira, São Paulo; Parque Estadual das Fontes do Ipiranga, São Paulo; and Reserva Biológica de Paranapiacaba, Santo André, which are all remnants of the Atlantic forest at São Paulo State, Southeast Brazil. When necessary, materials from other localities were examined.

Morphological study

The macroscopic descriptions and illustrations of basidiomata were based on fresh material. Colour terms are according to Küppers (1979). For microscopic analyses, the dried material was rehydrated in 70% ethanol followed by 5% KOH or Melzer's reagent. All microscopic illustrations were made with the aid of a drawing tube. The notation "[*a/b/c*]" at the beginning of a set of the basidiospores data is to be read "*a* spores were measured from *b* basidiomata taken from *c* collections", and the Q represents the range of length/width quotient for all spores measured. The basidiospores were measured in lateral view and the terms denoting its shape are according to Bas (1969). For the pileipellis cells, the pedicel measurement was given with the spheropedunculate cells and also separated, and a separate measurement was given when an apical projection was present. Generic and infrageneric concepts follow Singer (1958, 1986). The specimens and the voucher of the sequences are deposited at the Herbário do Estado Maria Eneyda P. Kauffmann Fidalgo (SP).

Molecular study

DNA extraction – Procedures for DNA extraction were done according to an adapted protocol by Zolan & Pukkila (1986) using lyophilised basidiomata previously ground to a fine powder in liquid nitrogen. The sample was resuspended in 50 µL of TE, incubated at 37°C for 30 min after the addition of RNase A (0.01 mg/µL), and stored at -20°C.

PCR amplification and DNA sequencing – The ITS + 5.8S region was amplified using the primer set ITS1-F and ITS4 (Vilgalys & Hester 1990; White *et al.* 1990; Gardes & Bruns 1993), and the nLSU rDNA was amplified using the primer set LR0R and LR5 (Moncalvo *et al.* 2000). PCR reaction, containing 2.0 U of Platinum® Taq DNA Polymerase (Invitrogen), 0.2 mM of dNTP mixture, 1.5 mM of MgCl₂, and 0.2 µM of each primer of the selected region in a total of 100 µL, was performed in a Eppendorf thermocycler, using the following program: 94°C for 5 min, 40 cycles at 94°C for 40 s, 55°C for 30 s, 72°C for 60 s, and 72°C for 5 min. Amplification products were purified using PureLink PCR Purification Kit (Invitrogen). DNA sequencing reactions were performed with the DYEnamic ET Dye Terminator Kit in a MegaBACE 1000 DNA sequencer (GE Healthcare) according to the manufacturer's instruction. The samples were sequenced in both directions. The sequences were deposited in GenBank (Table 1).

Table 1 – Collection data and GenBank accession number of the *Pluteus* taxa analysed.

Species	ITS + 5.8S	LSU	Voucher/ Strain	Reference
<i>Auricularia auricula-judae</i>	AF291268	-	MW 446	Weiß & Oberwinkler 2001
<i>A. polytricha</i>	-	AF261554	HN4076	Moncalvo <i>et al.</i> 2002
<i>Limacella glioderma</i>	AY176453	-	E.C. Vellinga 2456 (UC)	Vellinga 2004
<i>L. illinita</i>	-	AF261439	VT8.9.96	Moncalvo <i>et al.</i> 2002
<i>Pluteus admirabilis</i>	-	AF261577	DAOM193532	Moncalvo <i>et al.</i> 2002
	-	AF261578	DAOM197226	Moncalvo <i>et al.</i> 2002
<i>P. atromarginatus</i>	DQ494687	-	HKAS 31573	Matheny <i>et al.</i> 2006
	EF530926	-	UBC F16254	Denis <i>et al.</i> Not published
	-	AF261572	JB97/14	Moncalvo <i>et al.</i> 2002
<i>P. aurantiorugosus</i>	-	AF261579	DAOM197369	Moncalvo <i>et al.</i> 2002
<i>P. aureovenatus</i>	-	-	FK1045	This paper
<i>P. brunneoradiatus</i>	-	AF261567	JB97/3	Moncalvo <i>et al.</i> 2002
<i>P. capillicomptus</i>	-	-	NMJ128	This paper
	-	-	FK782	This paper
<i>P. cervinus</i>	-	AF261570	JB97/19	Moncalvo <i>et al.</i> 2002
<i>P. cervinus</i> var. <i>cervinus</i>	EU486448	-	UBC F16293	Denis & Berbee. Not published
<i>P. chrysophlebius</i>	-	AF261581	DAOM190194	Moncalvo <i>et al.</i> 2002
<i>P. dominicanus</i> var. <i>hyalinus</i>	-	-	FK1058	This paper
<i>P. ephebeus</i>	-	AF261574	JB97/23	Moncalvo <i>et al.</i> 2002
<i>P. fluminensis</i>	-	-	NMJ127	This paper
	-	-	FK1046	This paper
<i>P. fuligineovenosus</i>	-	-	FK826	This paper
<i>P. harrisii</i>	-	-	NMJ122	This paper
	-	-	FK1066	This paper
<i>P. jamaicensis</i>	-	-	NMJ130	This paper
<i>P. mammillatus</i>	-	DQ451549	ASM 7916	Minnis <i>et al.</i> 2006
<i>P. pallidus</i>	-	AF261569	JB90/27	Moncalvo <i>et al.</i> 2002
<i>P. pellitus</i>	-	AF261571	JB93/3	Moncalvo <i>et al.</i> 2002
<i>P. petasatus</i>	AF085495	-	CBS441.85	Lim & Jung 1998
	-	AF042611	JB91/21	Moncalvo <i>et al.</i> 2000
<i>P. pouzarianus</i>	-	AF261568	JB94/26	Moncalvo <i>et al.</i> 2002
<i>P. primus</i>	-	AF042610	JB94/24	Moncalvo <i>et al.</i> 2000
<i>P. ribertalensis</i> var. <i>conquistensis</i>	-	-	FK1043	This paper
<i>P. romellii</i>	AY854065	-	ECV 3201	Matheny <i>et al.</i> 2006
	-	AF261575	JB97/26	Moncalvo <i>et al.</i> 2002
<i>P. salicinus</i>	-	AF261573	JB97/6	Moncalvo <i>et al.</i> 2002
<i>Pluteus</i> sp.	-	AF261576	JMCR.124	Moncalvo <i>et al.</i> 2002
<i>Pluteus</i> sp.	-	AF042612	JM96/28	Moncalvo <i>et al.</i> 2000
<i>Pluteus</i> sp.	-	-	NMJ132	This paper
<i>P. sublaevigatus</i>	-	-	FK1085	This paper
<i>P. umbrosus</i>	-	AF261580	DAOM197235	Moncalvo <i>et al.</i> 2002
<i>P. xylophilus</i>	-	-	NMJ143	This paper
	-	-	NMJ147	This paper
<i>Volvariella volvacea</i>	AY636050	-	OE-140	Singh <i>et al.</i> 2003
	-	AF261531	Jmleg.SRL	Moncalvo <i>et al.</i> 2002

Data analysis

In order to elucidate the relationship of *Pluteus* species found in São Paulo State, Brazil we conducted phylogenetic analyses using nLSU and ITS + 5.8S sequences determined in this study with sequences available in GenBank (Table 1).

The sequences were analysed using BioEdit version 7.0.5.3 (Hall 1999) and then automatically aligned in Clustal W (Thompson *et al.* 1994). Parsimony analysis was performed with PAUP version 4.0b10 (Swofford 2003). Parsimony trees were obtained by heuristic searches with simple sequence addition in 1K replicates, employing tree-bisection-reconnection (TBR) branch-swapping algorithm. Characters from the extreme 5' and 3' ends of the sequences were deleted from all taxa to obtain individual datasets that had identical start and end positions, gaps were treated as missing, all characters were unordered and equally weighted, and multistate taxa was interpreted as uncertainty. Starting trees were obtained via stepwise addition, with one tree held at each step during stepwise addition, steepest descent option was not in effect. Also, the initial MaxTrees were set to auto-increase, branches of zero length were collapsed (creating polytomies), and MulTrees options were in effect.

Branch and branch node supports were determined using 1K BS replicates. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indexes) were determined. Trees generated were rooted to *Limacella illinita* (Fr.) Maire, *Auricularia polytricha* (Mont.) Sacc., and *Volvariella volvacea* (Bull.) Singer as the outgroup taxa for nLSU analyses; and *Auricularia auricula-judae* (Bull.) Quél., *Limacella glioderma* (Fr.) Maire, and *Volvariella volvacea* (Bull.) Singer for ITS + 5.8S analyses.

Results and discussion

Sequence data from nLSU region

In the nLSU analysis, 29 taxa plus 3 outgroups were aligned. The alignment dataset consisted of 1304 characters, including gaps. Prior to analysis, 515 characters from 5' and 3' ends of the sequences were excluded. Out of the 789 characters included in the analysis, 590 characters were constant, 109 variable characters were parsimony-uninformative and 90 were parsimony informative.

The heuristic searches with 1K BS replicates resulted in 24 equally most parsimonious (MP) tree, with the following scores: tree length = 380 steps, CI = 0.592, RI = 0.703, RC = 0.416, and HI = 0.408.

One MP tree with similar topologies generated from nLSU sequence data (Fig 1) revealed

a monophyletic clade with all *Pluteus* species (89% BS). Two clades can be distinguished with low BS support: one clade (58% BS) clustered species that belong to section *Pluteus*, and the other revealed a politomy including members of sections *Hispidoderma* and *Celluloderma* (51% BS).

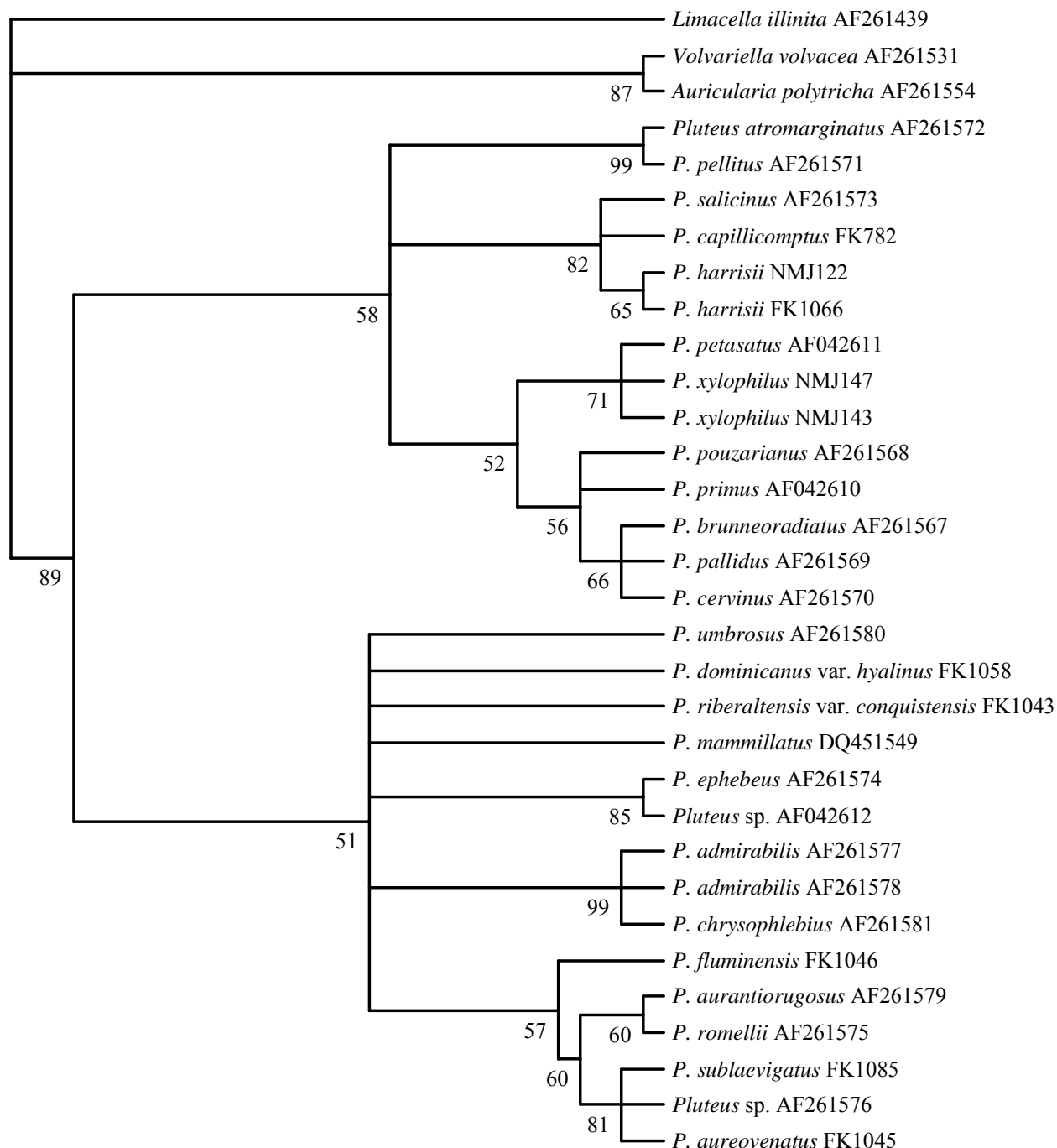


Fig 1 – Phylogenetic tree generated by MP analysis of partial LSU sequences. The tree is rooted using *Auricularia polytricha*, *Limacella illinita* and *Volvariella volvacea*. The BS numbers are shown before the nodes.

Sequence data from ITS + 5.8S region

In the ITS + 5.8S analysis, 19 taxa plus 3 outgroups were aligned. The alignment dataset

consisted of 1369 characters, including gaps. Prior to analysis, 884 characters from 5' and 3' ends of the sequences were excluded. Out of the 485 characters included in the analysis, 206 characters were constant, 81 variable characters were parsimony-uninformative and 198 were parsimony informative.

The heuristic searches with 1K BS replicates resulted in two equally MP trees, with the following scores: tree length = 725 steps, CI = 0.670, RI = 0.754, RC = 0.505, and HI = 0.330.

One MP tree with similar topologies generated from ITS + 5.8S sequence data also revealed a monophyletic clade with 64% BS support including all *Pluteus* species (Fig 2). This is divided into two clades; the upper including species from section *Celluloderma* with low BS support (55%), and the lower with high BS support (98%) clustering species from section *Pluteus*.

Phylogenetic analyses from rDNA sequence data

It was possible to confirm the monophyletic origin of *Pluteus* from both analyses (nLSU and ITS + 5.8S), as also shown by Moncalvo *et al.* (2002). Moreover, a separation tendency among *Pluteus* species into two clades was observed (Fig 1 and 2).

All species from section *Pluteus* clustered in the same clade (Fig 1 and 2), forming a well-supported group in accordance with the infrageneric classification of Singer (1958, 1986) who included in this section the species with metuloids pleurocystidia. The presence of *P. pallidus*, a species of section *Celluloderma*, in the section *Pluteus* clade (Fig 1), is probably due to a misidentification of the voucher material, since the species of the section *Celluloderma* always clustered in a different clade. The placement of *P. pallidus* was also verified by Moncalvo *et al.* (2002), however, the authors did not comment it, even though the section *Pluteus* was considered a well-supported group.

The clustering of species from section *Celluloderma* in one clade was verified in both analyses despite the low BS support (Fig 1 and 2). The nLSU analysis for section *Celluloderma* (Fig 1) showed a polytomy within a clade with low BS support (51% BS), including members of section *Hispiderma*. The relationship between members of section *Celluloderma* and section *Hispiderma* was also demonstrated by Moncalvo *et al.* (2002) in a well-supported clade (92% BS). Until now, it is not possible to recognise the monophyly of section *Hispiderma* and perhaps it represents an artificial group delimited by the morphological characters as proposed by Singer (1958, 1986). However, one should not disregard the possibility that the section *Hispiderma* is a subsection of *Celluloderma*, which

was proposed by Vellinga & Schreurs (1985). This future possibility requires the inclusion of other *Hispidoderma* species in the molecular analyses.

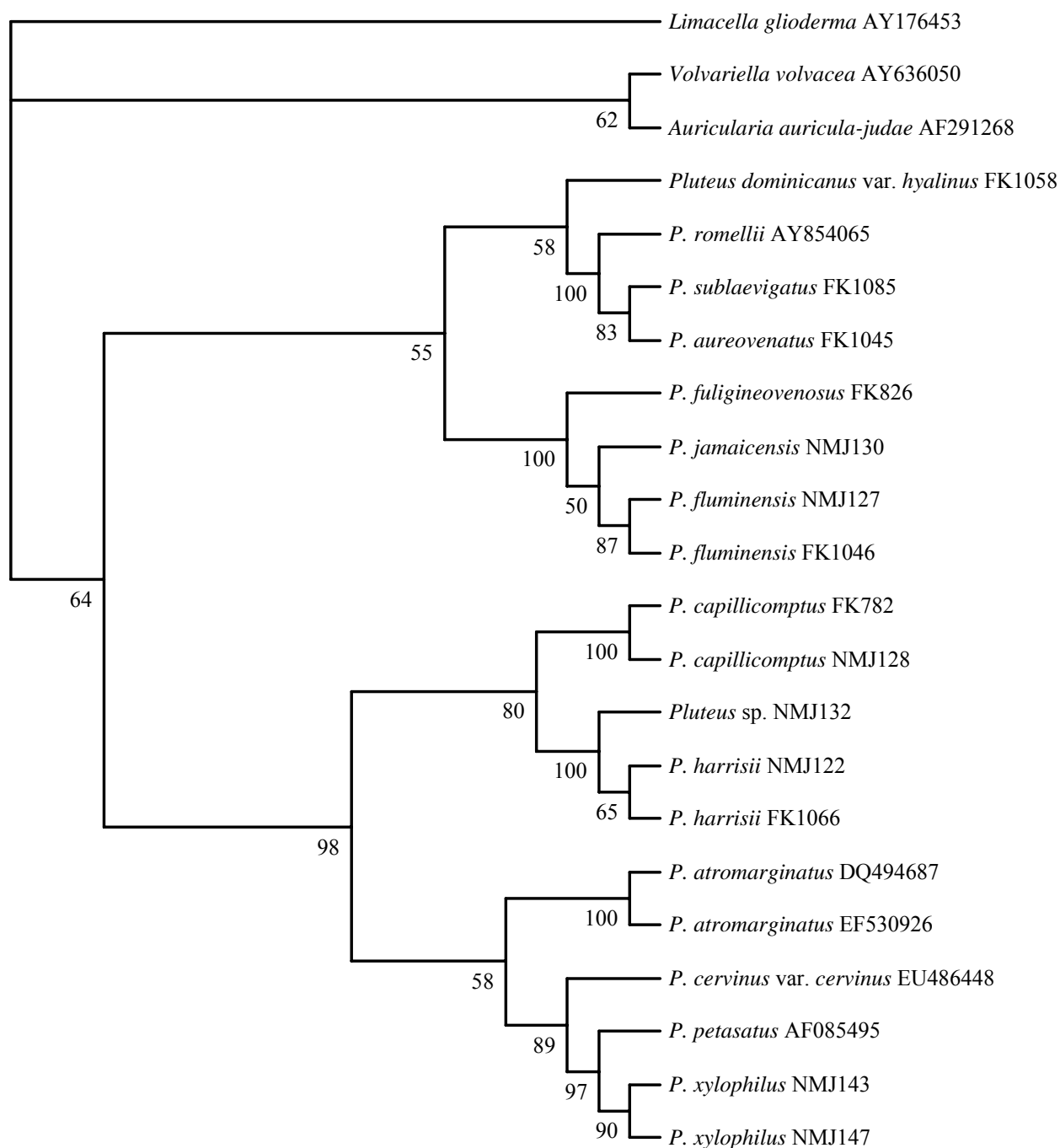


Fig 2 – Phylogenetic tree generated by MP analysis of ITS + 5.8S region. The tree is rooted using *Auricularia auricula-judae*, *Limacella glioderma* and *Volvariella volvacea*. The BS numbers are shown before the nodels.

In the ITS + 5.8S analysis (Fig 2), two clades can be distinguished within a major clade including only species of section *Celluloderma*. There is no relationship between these clades and the proposed subsections for section *Celluloderma*, since all analysed species are classified in the same subsection (*Eucellulodermini*). However, it can be inferred that some relationship exists between these species when pigmentation of the pileus and pileipellis is

taken into consideration. In this way, one clade (58% BS) including species with cream to yellowish-coloured pileus and hyaline or weakly pigmented pileipellis cells, and another well-supported clade (100% BS), including species with brownish pigment in the pileus and pileipellis cells, were distinguished.

The molecular analysis of Minnis *et al.* (2006) showed a close relationship between *P. mammillatus* and *P. ephebeus*, a member of section *Hispidoderma*. This fact was not confirmed in our analyses (Fig 1). However, based on the presence of thin-walled cystidia and the elongated hyphous elements of the pileipellis as described by Minnis *et al.* (2006), it is probable that *P. mammillatus* belongs to section *Hispidoderma* of Singer's classification (1958, 1986).

Taxonomy

Pluteus aureovenatus Menolli & Capelari, **sp. nov.** (Fig 3)

Etym.: The name refers to the colour and ornamentation of the pileus surface.

Pileus 22 mm diam., conicus vel convexus, aureoflavus, hygrophanus, venosus ad rugulosum, venae rubro-luteae, margo leviter sulcata, striata et leviter erosa. Lamellae subliberae, luteae. Stipes 30 × 1(apex)–3(basis) mm, curvus, leviter compressus, subbuliformis in basi, centralis, luteus. Basidiosporae 6.2(–7.5) × (5–)6.2(–7.5) μm, globosae, hyalinae, inamyloideae, laeves, crassis parietibus. Pleurocystidia (50–)56–68(–64) × (12.5–)15–22(–31) μm, clavata, hyalina, cum parietibus tenuibus. Cheilocystidia (25–)31–49(–61) × (8.7–)10–17(–21) μm, clavate vel versiformi-clavata, interdum leviter ventricosa, hyalina, cum parietibus tenuibus. Pileipellis cellularis a strato unico composita ex cellulis subglobosis, 54–70 × (36–)46–58 μm, hyalineis, absque pedicellis, interdum papilla apicali munitis. Fibulae absentes.

Typus: **Brazil**: *São Paulo State*: São Paulo City, Parque Estadual das Fontes do Ipiranga, 24 Jan. 2008 *F. Karstedt & L.A.S. Ramos 1045* (SP—holotypus).

Pileus 22 mm diam., conic to convex, deep yellowish orange, hygrophanous, veined-rugulose all over except at the margin, vein reddish-orange, margin slightly sulcate, translucently striate and slightly eroded. *Lamellae* sub-free, yellow, crowded, with lamellulae. *Stipe* 30 × 1(apex)–3(base) mm, curved, slightly compressed, with a subbulbous base, central, yellow, smooth, slightly longitudinally striate. *Basidiospores* [20/1/1] 6.2(–7.5) × (5–)6.2(–7.5) μm (Q = 1–1.25), globose, occasionally broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* 27–36 × 7.5–8.7 μm, clavate, slim, thin-walled, 4-spored. *Pleurocystidia* (50–)56–68(–64) × (12.5–)15–22(–31) μm, clavate, sometimes slightly fusoid,

frequently with apical mucilage in KOH, hyaline, thin-walled, moderately abundant. *Cheilocystidia* (25–)31–49(–61) × (8.7–)10–17(–21) µm, clavate to versiform-clavate, sometimes slightly ventricose, hyaline, thin-walled, moderately abundant. *Lamella edge* fertile. *Lamella trama* bilateral convergent, up to 44 µm width, composed of thin-walled hyphae, some commonly inflated, 2.5–18.7 µm diam., hyaline, septate, sometimes with oleiferous hyphae up to 3.7 µm diam. *Pileus context* undifferentiated, up to 230 µm thick, composed of thin-walled hyphae, 3.7–8.7 µm diam., hyaline, septate. *Pileipellis* cellular approximately 56 µm, composed of one layer of subglobose cells, 54–70 × (36–)46–58 µm, non-pedicelate, thin-walled, hyaline, occasionally with an apical extension digitate to papillate up to 20 µm long. *Clamp connections* absent in all parts examined.

Habitat and substrate. Solitary, on wood.

Comments. *Pluteus aureovenatus* is characterised by a deep yellowish-orange and veined pileus, conic to convex and margin translucently striate, lamellae sub-free and yellow, basidiospores globose, large non-pedicelate pileipellis cells, and pleuro- and cheilocystidia frequently clavate.

This species is closely related to *P. aurantiacus* Murrill, *P. aurantiorugosus* (Trog) Sacc. and *P. laetifrons* (Berk. & Curt.) Sacc. However, according to the type description (Murrill 1917) and Smith & Stuntz (1958), *P. aurantiacus* is different due to the pileus convex umbonate, basidiospores subglobose to broadly ellipsoid, pleuro- and cheilocystidia similar and slightly shorter [28–38(–50) × 9–14 × 5–10 µm] compared to *P. aureovenatus*, and cells of the pileipellis clavate-pedicellate and much smaller (15–33 µm). Singer (1956) considered *P. aurantiacus* as a synonym for *P. aurantiorugosus*. However, Smith & Stuntz (1958) recognized them as distinct species, since *P. aurantiorugosus* has an orange stipe and slightly longer and more elongated basidiospores. Regardless, *P. aurantiorugosus* differs from *P. aureovenatus* because, according to Homola (1972), *P. aurantiorugosus* has a stipe whitish to yellowish at first and then orange to red tints at the base, pileus of dried specimens orange to yellowish-orange instead of beige-straw for *P. aureovenatus*, basidiospores not predominantly globose (6–7 × 4.5–5 µm), and cells of the pileipellis vesiculose, clavate or occasionally subfusoid-ventricose and much shorter (22–39 × 22–31 µm) than *P. aureovenatus*.

Pluteus laetifrons is another species with orange-red pileus related to *P. aureovenatus*. However, Dennis (1953) and Singer (1958) described *P. laetifrons* with slightly smaller basidiospores (5–6 µm and 4.5–5.5 × 4.3–5.3 µm, respectively) and much smaller cells of the pileipellis (20–35 µm). Singer (1958) described a new variety of *P. laetifrons* from Bolivia,

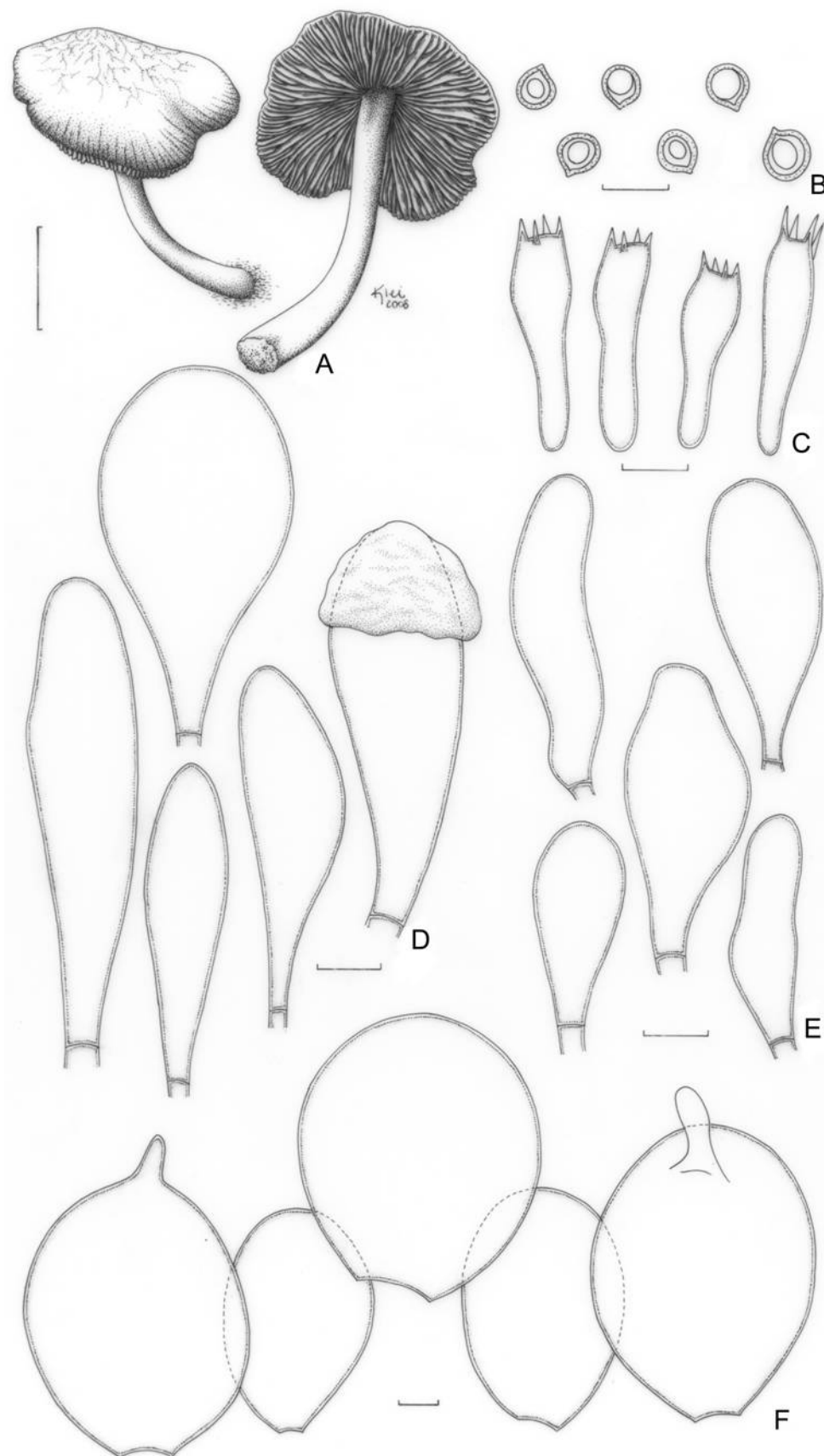


Fig 3 – *Pluteus aureovenatus* (holotype). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μ m.

viz. *P. laetifrons* var. *bolivianus* Singer, which differs from the type variety by larger and almost geometrically globose basidiospores ($5.3\text{--}6.8 \times 5.3\text{--}6.8 \mu\text{m}$) and darker deep coloured spots on the pileus which consist of pigmented pileipellis cells. Nevertheless, *P. aureovenatus* has pileipellis cells that are always hyaline and basidiospores that are occasionally broadly ellipsoid and slightly longer than those described for *P. laetifrons* var. *bolivianus*. Moreover, Singer (1958) did not mention the size of pileipellis cells for *P. laetifrons* var. *bolivianus*, that probably are $20\text{--}35 \mu\text{m}$, as described in the type variety, and therefore, much shorter than those observed for *P. aureovenatus*.

The relationship of *P. aureovenatus* with *P. aurantiacus*, *P. aurantiorugosus* and *P. laetifrons* is probably comparable to the relation between *P. chrysophlebius* and *P. sublaevigatus* (see discussion under *P. chrysophlebius*), where these species are separated by molecular data and have some micro-morphological differences such as pileipellis cell size and basidiospores that differ slightly in size and shape. *P. aureovenatus* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

***Pluteus capillicomptus* Menolli & Capelari, sp. nov.**

(Fig 4)

Etym.: The name refers to the arrangement of the fibrils in the pileus.

Pileus 34–37 mm diam., leviter vel distincter umbonatus, fusco-pallidus vel cinereo-fuscus, centro atrofusco, fibrillis radialiter appressis, margo sulcato-striata. Lamellae liberae, albae vel roseae. Stipes 35–35 \times 2(apex)–4(basis) mm, centralis, albus vel cremeo-pallidus, basis cinerea vel eburnea et pauco mycelio basali. Basidiosporae $(6.2\text{--})7.5\text{--}10(\text{--}11.2) \times (6.2\text{--})7.5\text{--}8.7 \mu\text{m}$, globosae sive ellipsoideae, hyalinae, rare subglobosae, laeves, parietibus crassis. Pleurocystidia dimorpha: typus I absque cornibus, $(39\text{--})50\text{--}83(\text{--}92) \times (11.2\text{--})15\text{--}24 \mu\text{m}$, cum apice sive subcapitato sive leviter strangulato; typus II similis *Pluteus harrisii* Murrill, bicornibus lateraliter, idem dimension vel minoribus quam typo I. Cheilocystidia $(25\text{--})31\text{--}52(\text{--}55) \times (6.2\text{--})8.7\text{--}13.7(\text{--}15) \mu\text{m}$, clavata, hyalina, cum parietibus tenuibus, interdum pedicello basi. Pileipellis repens. Fibulae absentes.

Typus: **Brazil**: *São Paulo State*: São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 24 Apr. 2007, *Menolli Jr. et al.* 128 (SP—holotypus).

Pileus 34–37 mm diam., plane, slightly to deeply umbonate, light brown to greyish brown ($N_{60}A_{50}M_{40}$ to $N_{80}A_{40}M_{50}$), darker with coffee shades at the centre ($N_{90}A_{50}M_{60}$), covered by appressedly and radially arranged fibrils, more concentrated at the centre and dissolving outside of central disc, finely pruinose at the centre, margin sulcate-striate. *Lamellae* free, white then pinkish, crowded, with lamellulae. *Stipe* 35–54 \times 2(apex)–4(base) mm, slightly

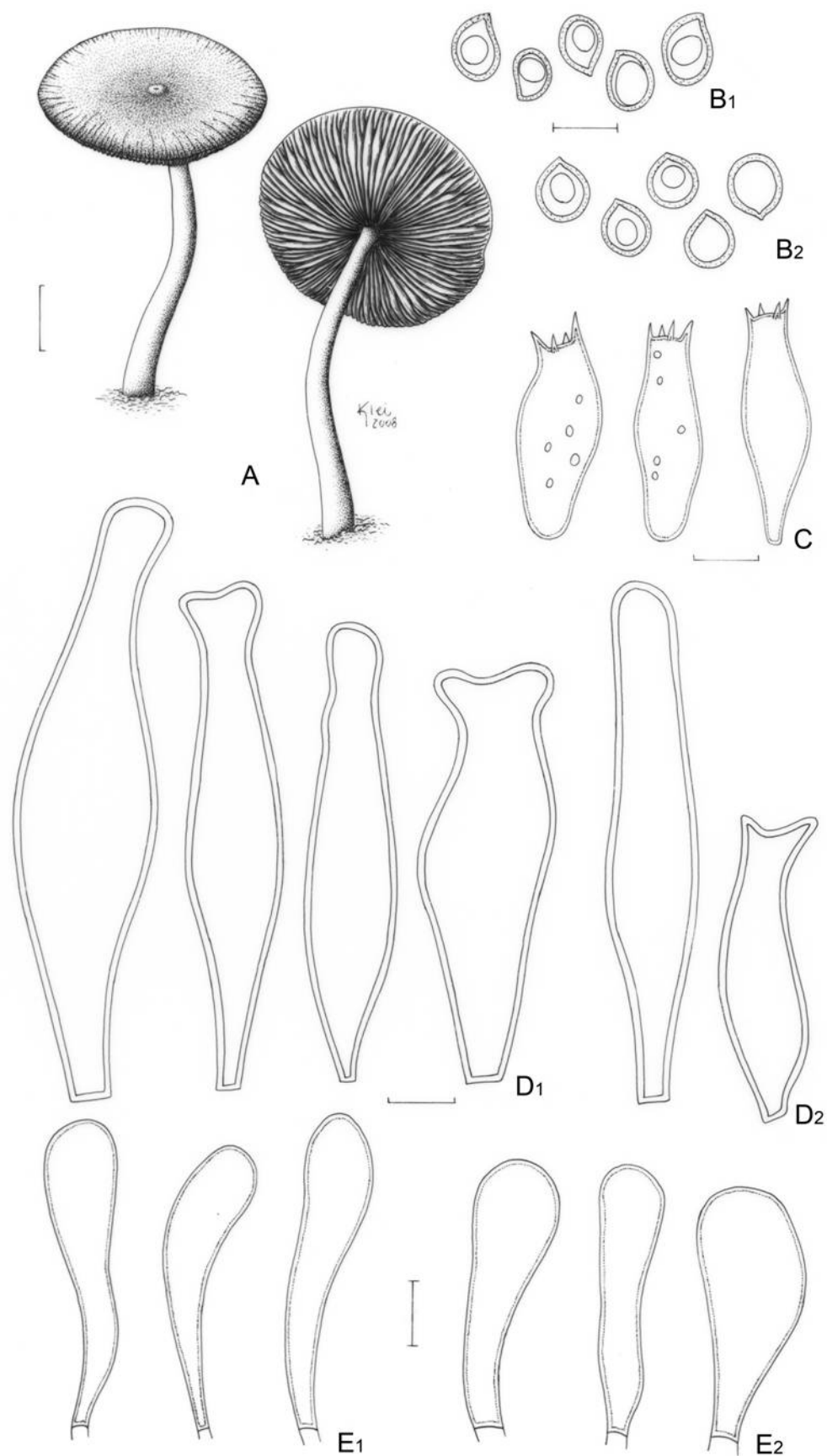


Fig 4 – *Pluteus capillicomptus*. (A) Basidioma (holotype). (B) Basidiospores; B₁. *F. Karstedt & M. Capelari* 782; B₂. holotype. (C) Basidia (holotype). (D) Pleurocystidia; D₁. *F. Karstedt & M. Capelari* 782; D₂. holotype. (E) Cheilocystidia; E₁. *F. Karstedt & M. Capelari* 782; E₂. holotype. Bars (A) = 1 cm; (B–E) = 10 µm.

tapering towards the apex, central, occasionally flexuous, white to pale cream with greyish to slightly silvery base, slightly longitudinally striate, with scanty basal mycelium. *Basidiospores* [40/2/2] (6.2–)7.5–10(–11.2) × (6.2–)7.5–8.7 μm [Q = 1–1.3(–1.4)], globose to broadly ellipsoid, occasionally subglobose or ellipsoid, inamyloid, hyaline, smooth, thick-walled, sometimes guttulate. *Basidia* (20–)25–31(–35) × 8.7–12.5 μm, versiform to clavate, thin-walled, 4-spored, sometimes with small scattered guttulae. *Pleurocystidia* of two types: (I) without prongs, (39–)50–83(–92) × (11.2–)15–24 μm, with a subcapitate or slightly strangulated at the apex, hyaline, sparse and moderately numerous; (II) most similar to those characteristic of *P. harrisii*, with two short lateral prongs, size similar to type I or rarely shorter in length with approximately 27 μm height, hyaline, very rare. In both types, the wall is uniform and slightly thickened up to 1 μm wide. *Cheilocystidia* (25–)31–52(–55) × (6.2–)8.7–13.7(–15) μm, clavate, rarely slightly ventricose, sometimes with a well developed pedicel at the base, hyaline, thin-walled, abundant. *Lamella edge* sterile. *Lamella trama* bilateral convergent, up to 62 μm width, composed of thin or slightly thick-walled hyphae, 2.5–10 (–12.5) μm diam., hyaline. *Pileus context* undifferentiated, thin, approximately 38 μm thick, composed of thin or slightly thick-walled hyphae, 3.7–10(–18.7) μm diam., hyaline. *Pileipellis* a repent epicutis up to 62 μm thick, composed of thin or slightly thick-walled hyphae, 2.5–6.2 μm diam., elongated, septate, with brown vacuolar content. *Clamp connections* absent in all parts examined.

Habitat and habit. Solitary, on wood.

Specimens examined. **Brazil.** *São Paulo State:* São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 24 Apr. 2007, Menolli Jr. et al. 128 (SP—holotype); 23 Oct. 2006, F. Karstedt & M. Capelari 782 (SP).

Comments. *Pluteus capillicomptus* is characterised by the light brown to greyish-brown plane pileus, deeply umbonate, and covered by appressedly and radially arranged fibrils; basidiospores with wide variation in size and shape; pleurocystidia of two types, usually without prongs or rarely with two short lateral prongs.

This species is related to *P. angustisporus* Singer and *P. harrisii* especially due to the presence of pleurocystidia without prongs or with poorly developed prongs. *Pluteus angustisporus* is quite similar to *P. capillicomptus* by the densely appressedly radially fibrillose pileus, with a slightly small umbo, but the first is described in the protologue (Singer 1958) with much smaller basidiospores [5.5–6(–7) × 2.7–3.7(–4.2) μm] and with very polymorphic pleuro- and cheilocystidia. *Pluteus harrisii* is different from *P. capillicomptus* because the first has pileus glabrous without appressedly fibrils (Murrill 1911; Singer 1956, 1958; Pegler 1983; Banerjee & Sundberg 1995).

Pluteus capillicomptus can be a transitional species between the sections *Hispidoderma* and *Pluteus* due to the presence of slightly thick-walled pleurocystidia with poorly developed prongs. However in the molecular analyses (Fig 1 and 2), *P. capillicomptus* clustered in a clade with all species of section *Pluteus*, including *P. harrisii*, with which it shares a relation. Therefore, *P. capillicomptus* is herein classified in the section *Pluteus*.

The two collections studied differ themselves in basidiospores and cheilocystidia shape (Fig 4). The holotype (Menolli Jr. et al. 128) has predominantly globose basidiospores and cheilocystidia usually clavate and without a long pedicel at base without. On the other hand, the collection *F. Karstedt & M. Capelari* 782 has basidiospores usually slightly ellipsoid and cheilocystidia frequently with a well developed pedicel at the base. However, the molecular analysis of ITS + 5.8S sequences (Fig 2) of both collections showed that they clustered with 100% BS. Thus, these characters probably constitute intraspecific variations that, with more collections to determine whether these characters are constant in successive fruitings, will be possible to verify if it is appropriate to attribute the varietal status to them.

Pluteus dominicanus var. *hyalinus* Menolli & Capelari, **var. nov.** (Fig 5)

Etym.: The varietal name refers to the hyaline pileipellis cells.

Similis *Pluteus dominicanus* var. *dominicanus* Singer sed cellulis hyalinis pileipellis differt.

Typus: **Brazil**: *São Paulo State*: São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 2. Jan.2008, *F. Karstedt et al.* 1058 (SP—holotypus).

Pileus 31 mm diam., plane-convex, slightly umbonate, beige to light brown, slightly rugose at the centre, margin translucently striate, slightly sulcate or sometimes eroded. *Lamellae* free, pinkish, subdistant, with lamellulae. *Stipe* 54 × 2(apex)–5(base) mm, tapering towards the apex, central, whitish to beige, smooth. *Basidiospores* [20/1/1] 6.2–7.5(–8.7) × 5–6.2(–7.5) μm (Q = 1.16–1.4), broadly ellipsoid, rarely ellipsoid, inamyloid, hyaline, smooth, thick-walled, sometimes guttulate. *Basidia* 20–29(–34) × 7.5–8.7 μm, clavate to versiform, thin-walled, 4-spored. *Pleurocystidia* (35–)42–61(–69) × (10–)11.2–16.2(–17.5) μm, slightly ventricose, lanceolate or sometimes fusiform-clavate, hyaline, sparse and few seen, thin-walled. *Cheilocystidia*, like the pleurocystidia, but smaller and very rare, (20–)29–43(–45) × (8.7–)10–14 μm. *Lamella edge* fertile. *Lamella trama* bilateral convergent, up to 50 μm width, composed of thin or slightly thick-walled hyphae, 2.5–7.5(–15) μm diam., hyaline. *Pileus context* undifferentiated, approximately 280 μm thick, composed of thin or slightly thick-walled hyphae, 3.7–7.5(–18.7) μm diam., hyaline. *Pileipellis* cellular up to 44 μm thick, composed of one layer of clavate to spheropedunculate cells, (26–)32–47(–53) × (13.7–)16.2–

21(–25) μm , with a short to long pedicel (6.2–25 μm long), thin-walled, hyaline. *Clamp connections* absent in all parts examined.

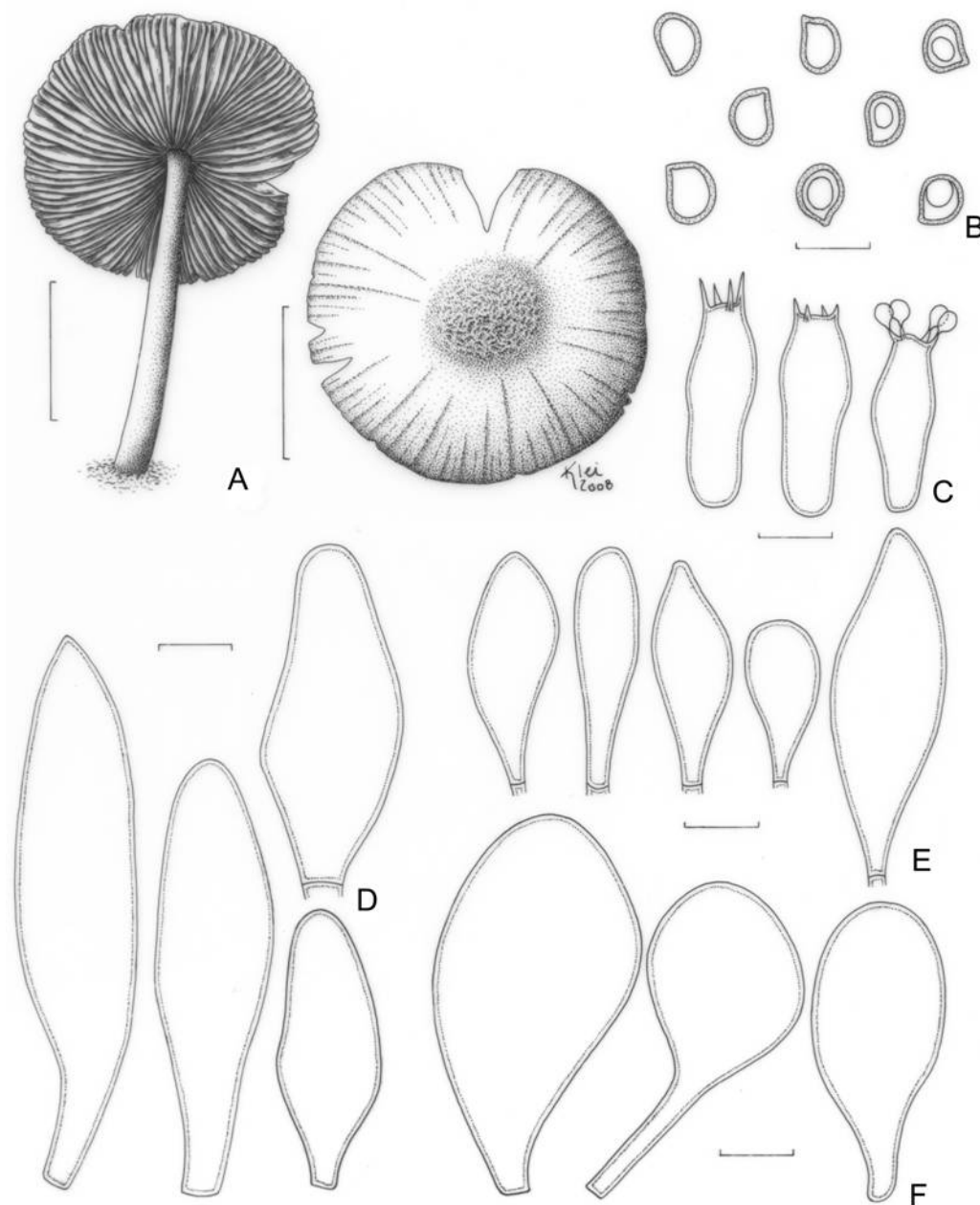


Fig 5 – *Pluteus dominicanus* var. *hyalinus* (holotype). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μm .

Habitat and habit. Solitary, on wood.

Comments. *Pluteus dominicanus* is known only from its type locality (Venezuela, Singer 1961) in its original description and subsequently recorded by Dennis (1970) based on the same material. *Pluteus dominicanus* var. *hyalinus* is slightly different from *P. dominicanus* var. *dominicanus* because Singer (1961) established the latter as “cheilocystidia not seen” and the pileipellis cells “with deep brownish-melleous dissolved intracellular pigment”, instead of

the cheilocystidia which is rarely seen and the hyaline pileipellis cells observed in Brazilian material. The re-examination of the type of *P. dominicanus* (K!, Fig 6) demonstrated that it has basidiospores broadly ellipsoid or occasionally subglobose, $7.5\text{--}8.7(-10) \times 6.2\text{--}7.5$ (-8.7) μm ; pleurocystidia slightly lanceolate to ventricose, $44\text{--}55 \times 15\text{--}19$ μm , cheilocystidia

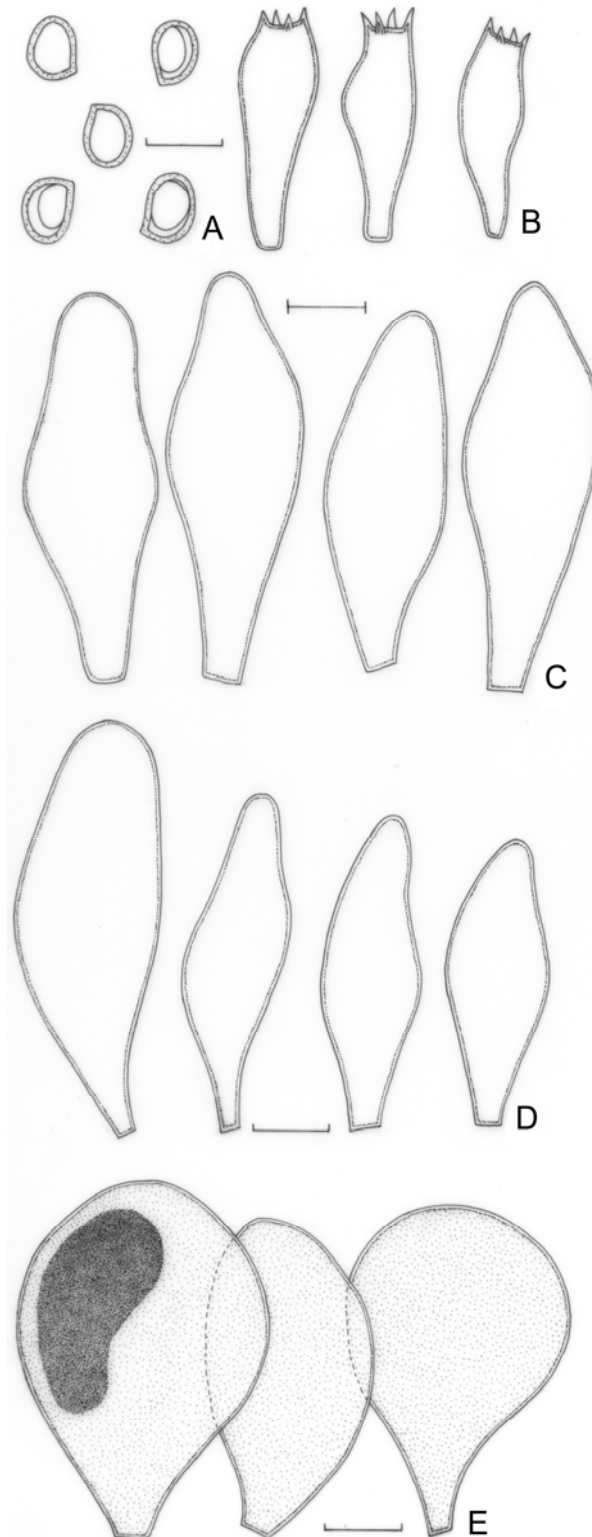


Fig 6 – *Pluteus dominicanus* (holotype). (A) Basidiospores. (B) Basidia. (C) Pleurocystidia. (D) Cheilocystidia. (E) Pileipellis cells. Bars = 10 μm .

present, $36\text{--}55 \times 12.5\text{--}20\text{--}(24)$ μm , like the pleurocystidia, but are very rare and only possible to see in tangential view; and pileipellis composed of vesiculose to espheropedunculate cells, $37\text{--}48\text{--}(57) \times 22\text{--}34\text{--}(39)$ μm , with a short to medium pedicel (2.5–10 μm long) and condensed or dissolved brownish pigment.

Pluteus dominicanus var. *hyalinus* is also related to the North American species *P. pallidus* and *P. umbrinodiscus*, but differs from these especially in the pileus colour and frequency of pleuro- and cheilocystidia. *Pluteus dominicanus* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

Pluteus fluminensis Singer, *Lloydia* **21**: 292 (1958). (Fig 7)

Pileus 27–31 mm diam., plane-convex, eventually slightly depressed at the centre or concave, not umbonate, deep sepia (N₈₀A₉₀M₆₀) to porphyry brown, darker brown and rugose to venose at the centre, slightly paler towards the margin, margin sulcate or finely cracking and showing the white flesh. *Lamellae* free to sub-free, white then pinkish, close, with lamellulae. *Stipe* 30–38 \times 2–3(apex)–5(base) mm, tapering towards the apex, with or without a small bulb, central, hollow, pale cream slightly greyish or translucent, finely pruinose and with finely brownish fibrils at base, slightly longitudinally striate, with scanty basal mycelium. *Basidiospores* [100/4/4] 6.2–7.5 \times 5–6.2 μm (Q = 1.2–1.24), broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* 21–37 \times 7.5–8.7(–10) μm , clavate to versiform, sometimes more slender, thin-walled, 4-spored. *Pleurocystidia* (41–) 47–65(–69) \times 12.5–21(–25) μm , ventricose or slightly ventricose to lageniform, rarely vesiculose-saccate, hyaline, thin-walled, sparse, moderately abundant to scattered. *Cheilocystidia* (25–)32–50(–56) \times (10–)11.2–18.7 (–22) μm , like the pleurocystidia or rarely subclavate to clavate, difficult to see, moderately numerous. *Lamella edge* heteromorphous. *Lamella trama* bilateral convergent, up to 62 μm width, composed of thin or slightly thick-walled hyphae, 1.2–8.7(–15) μm diam., hyaline, septate. *Pileus context* undifferentiated, up to 125 μm thick, composed of thin or slightly thick-walled hyphae, 3.7–5 μm diam., hyaline, septated, sometimes with oleiferous up to 3.7 μm diam. *Pileipellis* cellular approximately 45 μm thick, composed of one layer of subglobose, vesiculose or subclavate cells, (20–)25–51 (–61) \times (17.5–)20–35(–42) μm , frequently non-pedicelate, often with a short to medium pedicel (1.2–7.5 μm long), thin-walled, with condensed or dissolved brown pigment. *Clamp connections* absent in all parts examined.

Habitat and substrate. Solitary or in pairs, on wood.

Specimens examined. **Brazil:** *Rio de Janeiro State:* Angra dos Reis, 31 Sep.1953, R. Singer B432 (F—holotype); *São Paulo State:* Cananéia, Ilha do Cardoso, between Morro Três

Irmãos and Sítio Grande, 23 Oct. 1984, *M. Capelari 164* (SP); near Morro Três Irmãos, 18 Dec. 1984, *M. Capelari & R. Maziero 212* (SP); São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 24 Apr. 2007, *Menolli Jr. et al. 127* (SP); 24 Jan. 2008, *F. Karstedt & L.A.S. Ramos 1046* (SP).

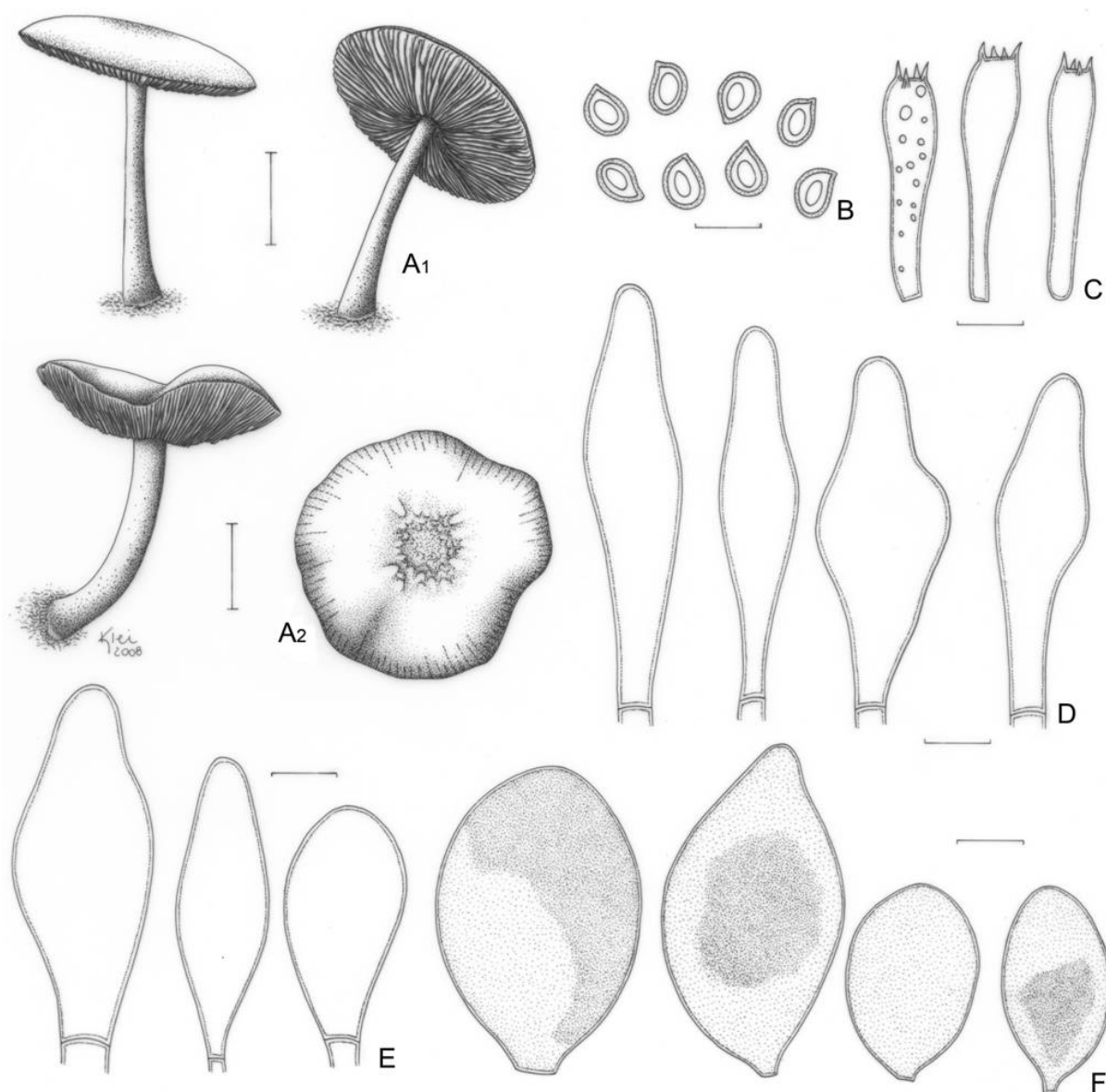


Fig 7 – *Pluteus fluminensis*. (A) Basidioma; A₁, *Menolli Jr. et al. 127*; A₂, *F. Karstedt & L.A.S. Ramos 1046*. (B–F) *F. Karstedt & L.A.S. Ramos 1046*. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μ m.

Comments. *Pluteus fluminensis* was described from Brazil and also reported for Bolivia and U.S.A (Florida) in its original description (Singer 1958). Later, Pegler (1997) reported it for São Paulo State and Stijve & Meijer (1993) and Meijer (2006) for Paraná State as *P. cf. fluminensis*. The basidiospores of *P. fluminensis* were described in the protologue (Singer 1958) as “subglobose or rarely a few piriform, (4.5–)5–6.3(–7) \times (4–)4.3–5.3(–5.8)

µm”, however, upon re-examination of the type material, it was demonstrated that the basidiospores are broadly ellipsoid, measuring $6.2\text{--}7.5 \times 5.0\text{--}6.2$ µm. The pleuro- and cheilocystidia of the type are probably ventricose, but they are collapsed and it was not possible to confirm the size and shape. *Pluteus jamaicensis* is closely related to *P. fluminensis*, but according to Singer (1958) the first has epicuticular elements with dissolved internal pigment, instead of pigment condensations as observed in *P. fluminensis*. The occurrence of subglobose to vesiculose pileipellis cells with condensed brown pigment was confirmed in the re-examination of the type and in additional collections of *P. fluminensis* herein studied. Singer (1958) also considered the future possibility of subspecies status for *P. fluminensis* in *P. jamaicensis*. However, it is herein considered two distinctive species also supported by molecular analysis (Fig 2). *Pluteus fluminensis* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

Pluteus fuligineovenosus Horak, *Nova Hedwigia* **8**: 190 (1964). (Fig 8)

Pileus 14–38 mm diam., plane to slightly conic, apparently subumbonate, dark brown, slightly paler towards the margin and darker to blackish at the centre, veined all over especially around the centre then radially arranged towards the margin, vein darker, margin apparently neither sulcate nor striate. *Lamellae* free to slightly sinuate, white then pinkish, subdistant, with few lamellulae. *Stipe* 8–31 × 1–4 mm, slightly tapering towards the apex, central to slightly excentric, slightly flexuous, apparently hollow, translucent white, slightly longitudinally striate, with scanty basal mycelium. *Basidiospores* [20/1/1] $6.2\text{--}7.5 \times 5\text{--}6.2$ (–7.5) µm (Q = 1–1.25), globose, occasionally broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* $18.7\text{--}21$ (–25) × $6.2\text{--}7.5$ µm, clavate to versiform, thin-walled, 4-spored. *Pleurocystidia* (51–)57–77 × 11.2–18.7 µm, fusoid-ventricose to lanceolate, hyaline, thin-walled, sparse and moderately abundant. *Cheilocystidia* (37–)45–63 × (12.5–)15–20 µm, clavate to elongate-clavate, hyaline, thin-walled, not numerous. *Lamella edge* apparently sterile. *Lamella trama* bilateral convergent, up to 31 µm width, composed of thin or slightly thick-walled hyphae, 3.7–11.2 µm diam., hyaline, few septa seen, with oleiferous hyphae up to 5 µm diam. *Pileus context* undifferentiated, approximately 47 µm thick, composed of thin or slightly thick-walled hyphae, 5–7.5 µm diam., hyaline, few septa seen. *Pileipellis* cellular up to 100 µm, composed of one layer of vesiculose to spheropedunculate cells, (32–)39–50 (–62) × (17.5–)20–32(–39) µm, with a short to long pedicel (2.5–23 µm long), thin-walled, with condensed or dissolved brown to chestnut-brown pigment. *Clamp connections* absent in all parts examined.

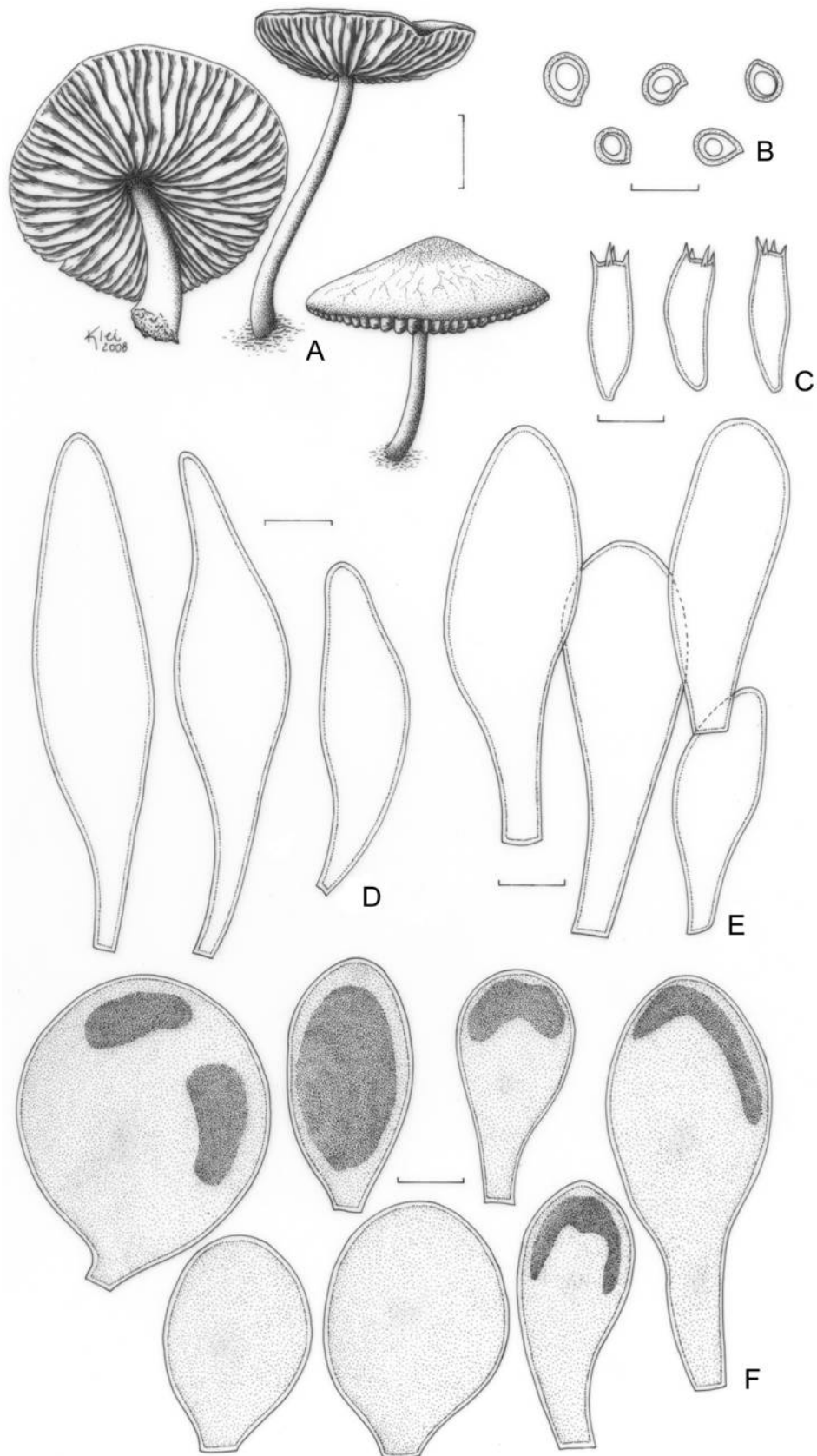


Fig 8 – *Pluteus fuligineovenosus* (F. Karstedt & M. Capelari 826). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μ m.

Habitat and substrate. Gregarious to disperse, on wood.

Specimens examined. **Brazil:** São Paulo State: São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 12 Dec. 2006, F. Karstedt & M. Capelari 826 (SP).

Comments. *Pluteus fuligineovenosus* is characterised by the brown and veined pileus, with darker to blackish centre and veins, basidiospores globose, and spheropedunculate cells of the pileipellis with condensed or dissolved brown to chestnut-brown pigment.

This species is only known from Chile (Horak 1964). The Brazilian collection has basidiospores slightly bigger than that mentioned in the original description ($5.6\text{--}6.6 \times 5.2\text{--}5.6 \mu\text{m}$); pleurocystidia more frequently fusoid ventricose; stipe apparently hollow; and scanty micelium at the base, instead of solid and lacking basal micelium in the Chilean material. This work reports the first record of *P. fuligineovenosus* for Brazil and the second collection of the species since its description. *Pluteus fuligineovenosus* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

Pluteus harrisii Murrill, *Mycologia* **3**: 277 (1911). (Fig 9)

Pileus 20–33(–55) mm diam., conic to convex, slightly umbonate, hazel (N₆₀A₈₀M₇₀) to brown or with coffee shades, slightly darker at the centre, with or without finely white punctuation especially at the centre, margin finely sulcate to sulcate-striate. *Lamellae* free, white then pinkish, attachment partially unequal, crowded, with lamellulae. *Stipe* 30–48(–62) × 2(apex)–5(base) mm, subequal, central, occasionally flexuous, apparently hollow, white to whitish grey, slightly pruinose at apex, longitudinally striate, with basal mycelium. *Basidiospores* [80/4/4] 7.5–8.7(–10) × (5–)6.2–7.5(–8.7) μm (Q = 1.14–1.5), broadly ellipsoid to ellipsoid, rarely subglobose, inamyloid, hyaline, slightly pinkish in KOH, smooth, thick-walled, sometimes guttulate. *Basidia* 24–30 × 7.5–10 μm, versiform to clavate, thin-walled, 4-spored, sometimes with small scattered guttulae. *Pleurocystidia* (40–)52–82(–91) × 15–24 μm, fusoid-ventricose, hyaline, sparse, moderately numerous to abundant, moderately thick-walled, wall usually uniform up to 1.2 μm wide or sometimes collapsing towards the base and thickening up to 2.5 μm wide at the apex, apices usually with 2–4 versiform lateral prongs (up to 5 μm long), prongs rarely with a secondary bifurcation, occasionally without prongs and with a rounded or deformed apex, sometimes a colourless internal condensed content is present. *Cheilocystidia* (31–)36–59 × 8.7–15(–17.5) μm, clavate to versiform-clavate, usually with a moderately long pedicel, hyaline, moderately abundant, thin-walled. *Lamella edge* sterile. *Lamella trama* bilateral convergent, up to 62 μm width, composed of thin or slightly thick-walled hyphae, 2.5–13.7 μm diam., sometimes branched or with terminal elements slightly inflated up to 17.5 μm diam., hyaline, septate. *Pileus context* undifferentiated, up to

200 μm thick, composed of thin or slightly thick-walled hyphae, 3.7–8.7 μm diam., sometimes inflated up to 25 μm diam., hyaline, septate. *Pileipellis* a repent epicutis, up to 100

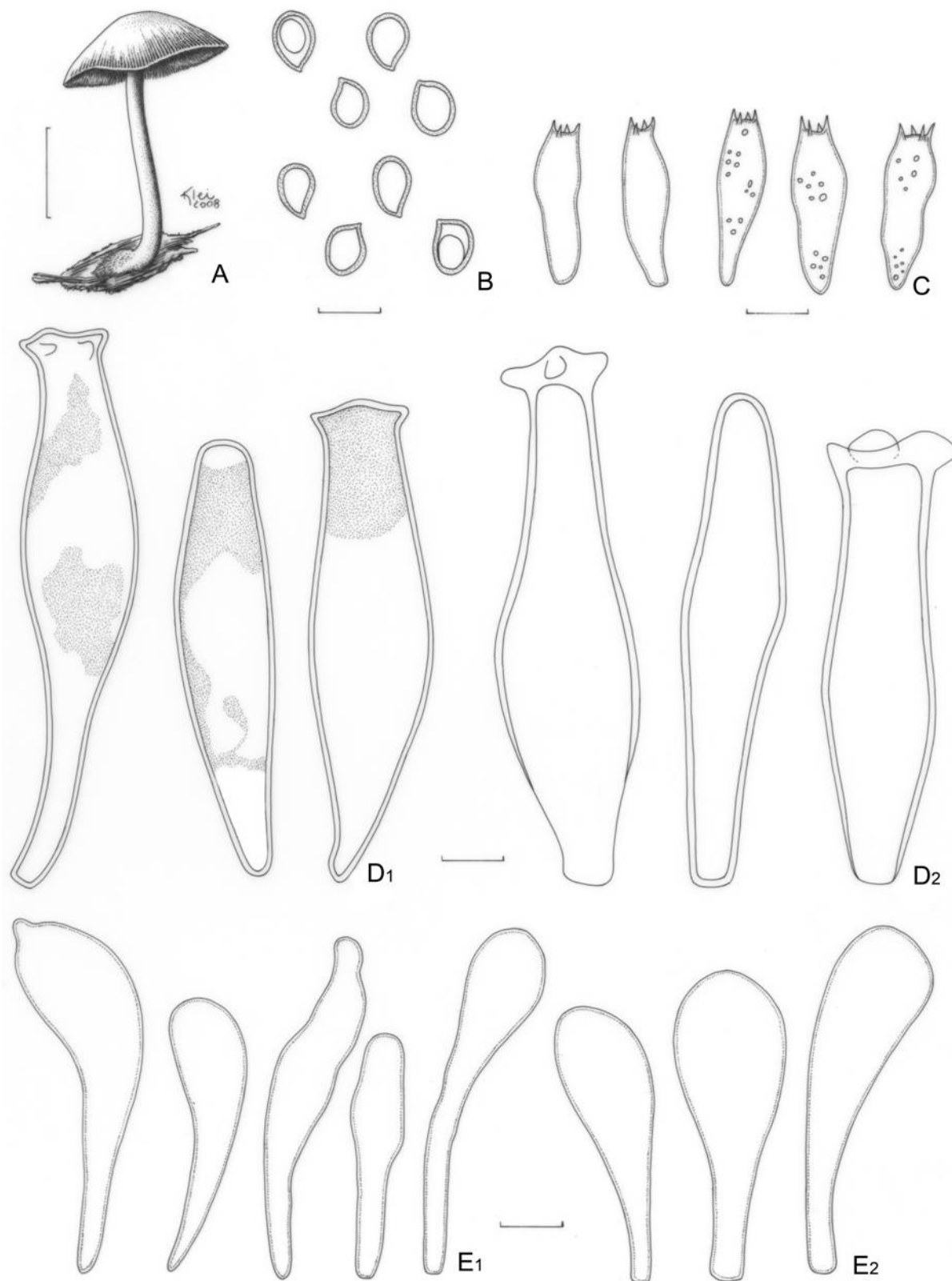


Fig 9 – *Pluteus harrisii*. (A–C) Menolli Jr. et al. 122. (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia; D₁. Menolli Jr. et al. 122; D₂. F. Karstedt et al. 1066. (E) Cheilocystidia; E₁. Menolli Jr. et al. 122; E₂. F. Karstedt et al. 1066. Bars (A) = 1 cm; (B–E) = 10 μm .

µm thick, composed of thin to slightly thick-walled hyphae, 5–12.5 µm diam., elongated, septate, with light to dark brown content, sometimes with terminal elements slightly inflated. *Clamp connections* absent in all parts examined.

Habitat and substrate. Solitary, on leaf of palm tree or decaying wood.

Specimens examined. **Brazil:** *São Paulo State:* São Miguel Arcanjo, Parque Estadual Carlos Botelho, 25 Apr. 1986, M. Capelari & V.L.R. Bononi 10 (SP); Santo André, Reserva Biológica de Paranapiacaba, 11 Apr. 1990, M. Capelari et al. 3282 (SP); 22 March 2007, Menolli Jr. et al. 122 (SP); São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 31 Jan. 2008, F. Karstedt et al. 1066 (SP).

Comments. *Pluteus harrisii* is characterised by the dark brown pileus and white stipe with greyish tones, and the metuloids with poorly developed apical prongs. This species was originally described from Jamaica by Murrill (1911), who established *P. harrisii* without cystidia. However, Singer (1956, 1958), Pegler (1983), and Banerjee & Sundberg (1995) found thick-walled pleurocystidia with short apical prongs in the holotype.

Besides the type locality, *P. harrisii* was also recorded from Cuba (Murrill 1911), Trinidad (Baker & Dale 1951 as “*P. cervinus* var. *bambusinus*”), U.S.A – Florida (Singer 1956, 1958), Guadeloupe (Pegler 1983), Mexico (Vargas 1993; Rodríguez & Guzmán-Dávalos 2001), and Brazil (Pegler 1997; Meijer 2006 as *P. aff. harrisii*).

The Brazilian material studied has basidiospores moderately larger than those found by Singer (1956, 1958) and pleurocystidia sometimes with a hyaline amorphous substance as recorded by Banerjee & Sundberg (1995) in the type revision. However, it has a stipe apparently hollow instead of a solid stipe as recorded by Singer (1956, 1958) and Pegler (1983).

Two of the studied collections, Menolli Jr. et al. 122 and F. Karstedt et al. 1066, have the same nLSU sequence and only differ in two base pairs in the ITS + 5.8S sequence. However, Menolli Jr. et al. 122 differ by the presence of a colourless internal condensed content in the pleurocystidia and fewer cheilocystidia that are usually versiform. It will be necessary to study more collections to determine whether these characters are constant in successive fruitings in order to verify if they constitute intraspecific variations or are sufficient to consider them as independent taxa. *Pluteus harrisii* belongs to section *Pluteus*.

Pluteus jamaicensis Murrill, *Mycologia* **3**: 278 (1911).

(Fig 10)

Pileus 17–34 mm diam., campanulate-convex, sometimes slightly umbonate, dark beige to dark brown (N₇₀A₈₀M₇₀), slightly paler towards the margin, finely rivulose all over, rugose to rivulose at the centre, margin slightly sulcate and sometimes finely cracking and showing the

white flesh. *Lamellae* free to sinuate-remote, white then pinkish, with numerous lamellulae. *Stipe* 20–30 × 1–2 mm, equal, central, hollow, cream to translucent white, slightly greyish at base. *Basidiospores* [20/1/1] 6.2–7.5 × 5–6.2 μm (Q = 1.2–1.25), broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, sometimes guttulate. *Basidia* 25–29(–31) × 6.2–8.7 μm, clavate to versiform, thin-walled, 4-spored. *Pleurocystidia* (46–)50–56 × 10–13.7 (–17.5) μm, fusoid-ventricose to lanceolate, hyaline, thin-walled, sparse and not abundant.

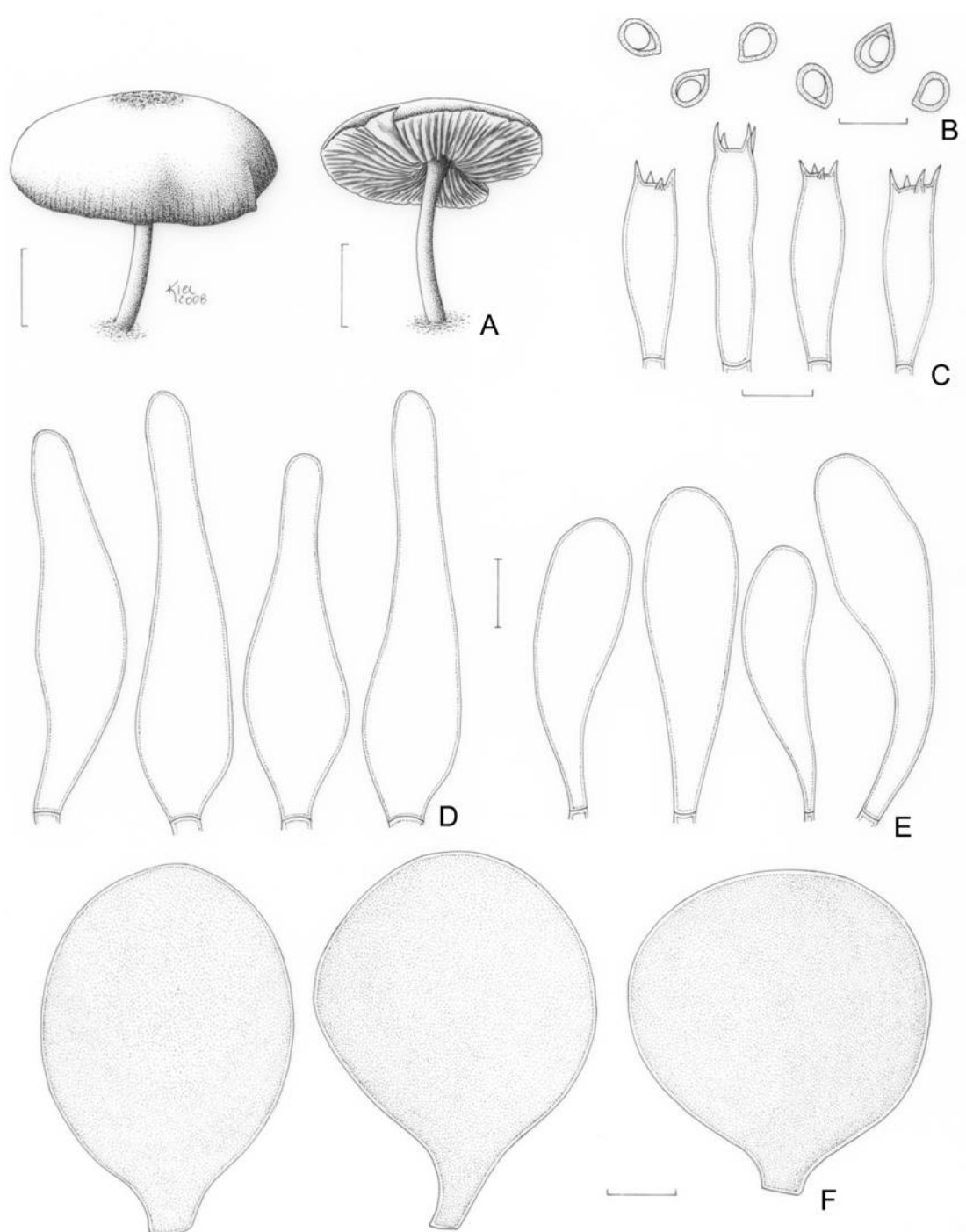


Fig 10 – *Pluteus jamaicensis* (Menolli Jr. et al. 130). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μm.

Cheilocystidia (25–)31–45(–50) × (7.5–)10–12.5 µm, clavate to elongate-clavate, hyaline, thin-walled, numerous. *Lamella edge* heteromorphous with few basidia. *Lamella trama* bilateral convergent, up to 62 µm width, composed of thin or slightly thick-walled hyphae, 3.7–13.7 µm diam., hyaline. *Pileus context* undifferentiated, approximately 62 µm thick, composed of thin or slightly thick-walled hyphae, hyaline, 3.7–10 µm diam. *Pileipellis* cellular up to 125 µm, composed of one or more layers of spheropedunculate cells, (37–)45–54 × (31–)37–42 µm, with a short pedicel (5–10 µm long), thin-walled, with dissolved brown cytoplasmic content. *Clamp connections* absent in all parts examined.

Habitat and substrate. Gregarious, on wood.

Specimens examined. **Brazil:** São Paulo State: São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 24 Apr. 2007, *Menolli Jr. et al.* 130 (SP).

Comments. *Pluteus jamaicensis* was described from Jamaica (Murrill 1911) and since then it was reported from Trinidad (Dennis 1953 as *P. aethalus* var. *jamaicensis*; Pegler 1983), Argentina (Singer & Digilio 1952 as “*P. phlebophorus* var. ?”; Singer 1956, 1958), Venezuela ? (Dennis 1970), Martinique, and Guadalupe (Pegler 1983). Besides, according to Singer (1958), *P. phlebophorus* (Ditmar) P. Kumm. *sensu* Rick (1938) reported for São Leopoldo, Rio Grande do Sul State, Brazil is perhaps *P. jamaicensis*. The species was originally described without cystidia (Murrill 1911), but posterior revisions of the type confirmed the presence of marginal and facial cystidia (Singer 1958; Smith & Stuntz 1958; Pegler 1983; Banerjee & Sundberg 1993). The Brazilian collection has pileus margin slightly sulcate as reported by Singer (1956, 1958) from Argentinean materials; cheilocystidia preponderantly clavate as described by Pegler (1983); and basidiospores broadly ellipsoid and slightly longer than those described by Smith & Stuntz (1958) in the revision of the type specimen (subglobose, 5–6 × 5–5.5 µm). *Pluteus fluminensis* is closely related to *P. jamaicensis*, however, herein they are considered two distinctive species (see discussion under *P. fluminensis*). This work is the first record of *P. jamaicensis* for Brazil. *Pluteus jamaicensis* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

Pluteus riberaltensis* var. *conquistensis Singer, *Lloydia* **21**: 255 (1911). (Fig 11)

Pileus 27–53 mm diam., campanulate when young, then convex, umbonate, dark brown, slightly darker in the centre, slightly radially coloured, finely pruinose all over and finely cracking especially around the centre, margin not striate and showing the whitish flesh between the fibrils. *Lamellae* free, pinkish, moderately crowded, with lamellulae. *Stipe* 53–65 × 4–9 mm, slightly tapering towards the apex, base subbulbous, central to slightly excentric, white-cream with brown fibrils over the surface especially when young, then brownish and

slightly longitudinally striate, with scanty mycelium at base. *Basidiospores* [20/1/1] $6.2\text{--}7.5 \times 6.2 \mu\text{m}$ ($Q = 1\text{--}1.2$), globose, occasionally broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* $21\text{--}27(\text{--}31) \times (7.5\text{--})8.7\text{--}10 \mu\text{m}$, clavate, thin-walled, 4-spored, with small scattered guttulae. *Pleurocystidia* $(44\text{--})48\text{--}69(\text{--}78) \times (15\text{--})17.5\text{--}28(\text{--}35) \mu\text{m}$, slightly ventricose to lageniform, sparse and not abundant. *Cheilocystidia* $(27\text{--})30\text{--}47(\text{--}57) \times (12.5\text{--})17.7\text{--}27 \mu\text{m}$, like pleurocystidia, but very rooting on the hymenium and hard to see. *Lamella edge* fertile with abundant basidia. *Lamella trama* bilateral convergent, up to $37 \mu\text{m}$ width, composed of thin or thick-walled hyphae, $3.7\text{--}10 \mu\text{m}$ diam., hyaline, few septa seen, with oleiferous hyphae up to $3.7 \mu\text{m}$ diam. *Pileus context* undifferentiated, approximately $125 \mu\text{m}$ thick, composed of thin or thick-walled hyphae, $3.7\text{--}7.5 \mu\text{m}$ diam., hyaline, septate. *Pileipellis* a cutis, approximately $100 \mu\text{m}$ thick, composed of thin-walled hyphae, $10\text{--}20 \mu\text{m}$ diam., elongated, septate, with dissolved brown vacuolar content, sometimes with the terminal elements slightly inflated and often ascendant. *Clamp connections* absent in all parts examined.

Habitat and substrate. In pairs on wood.

Specimens examined. **Brazil.** *São Paulo State:* São Paulo City, Parque Estadual das Fontes do Ipiranga, 24 Jan. 2008, *F. Karstedt & L.A.S. Ramos 1043* (SP).

Comments. This variety was described from Bolivia (Singer 1958) and differs from the type variety almost exclusively by the umber-grey fibrils on stipe. The Brazilian specimen is characterised by brown fibrils over the stipe surface especially when young as described for this variety. Also, there are some cavities on the pileus surface, probably caused by insects, were observed showing the flesh. However, our material has pleurocystidia moderately longer than those described for *P. riberaltensis* var. *conquistensis* ($38\text{--}41 \times 14\text{--}22.5 \mu\text{m}$) and these are more similar to those the type variety ($41\text{--}86 \times 12.5\text{--}39 \mu\text{m}$) as described in the protologue (Singer 1958).

Later, Singer (1961) described another variety, viz. *P. riberaltensis* var. *missionensis* Singer, based on an Argentinean specimen which has a white stipe similar to the type variety, but differs from the latter by the conspicuous incrustation of cystidia, and the same size of cheilo- and pleurocystidia. *Pluteus riberaltensis* and its varieties were described based on single collections and since then no other records have been reported. This work presents the first mention of *P. riberaltensis* var. *conquistensis* for Brazil and the second record since its description. *Pluteus riberaltensis* var. *conquistensis* belongs to section *Hispidoderma*.

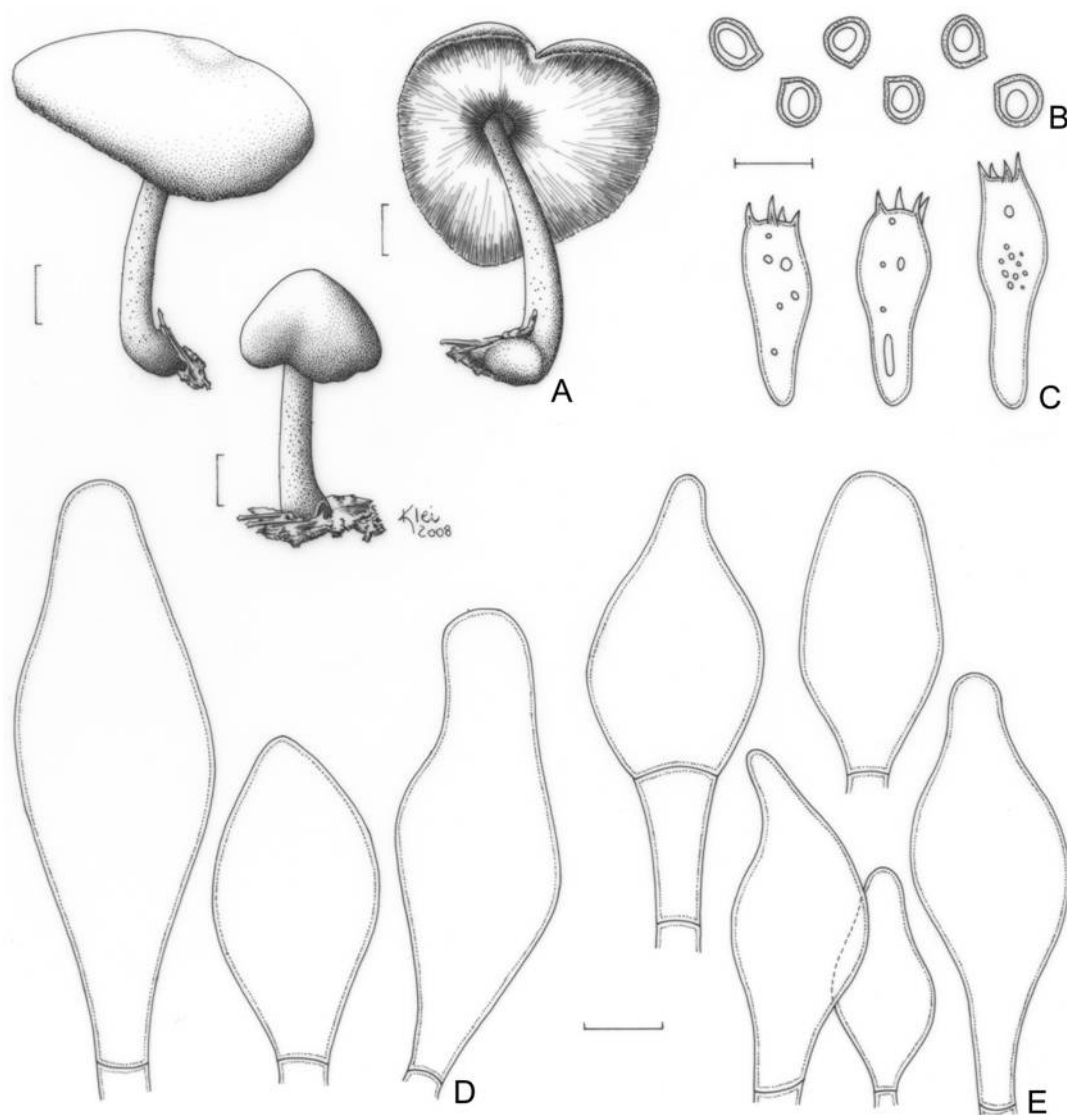


Fig 11 – *Pluteus riberalensis* var. *conquistensis* (F. Karstedt & L.A.S. Ramos 1043). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. Bars (A) = 1 cm; (B–E) = 10 μ m.

***Pluteus sublaevigatus* (Singer) Menolli & Capelari, stat. nov.**

(Fig 12)

Basionym: *Pluteus chrysophlebius* subsp. *sublaevigatus* Singer, *Lloydia* **21**: 278 (1958).

Pileus 11–28 mm diam., campanulate when young, then convex to plane, not umbonate, sometimes slightly depressed at the centre, yellow (N₀₀A₃₀M₄₀ to N₀₀A₈₀M₃₀), hygrophanous, slightly rugulose at the centre, margin translucently striate, sometimes eroded. *Lamellae* free to subfree, close, yellowish, moderately crowded, with lamellulae. *Stipe* 28–33 × 1–4 mm, curved, cylindric with a subbulbous base, central, whitish yellow, more yellowish at the base, surface smooth, sometimes with white punctuations and white pruinose mycelium at the base. *Basidiospores* [60/3/2] (5–)6.2–7.5 × 5–7.5 μ m (Q = 1–1.25), preponderantly globose, sometimes broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate, sometimes with small scattered guttulae. *Basidia* (20–)22–27(–30) × 7.5–8.7(–10) μ m, clavate, thin-

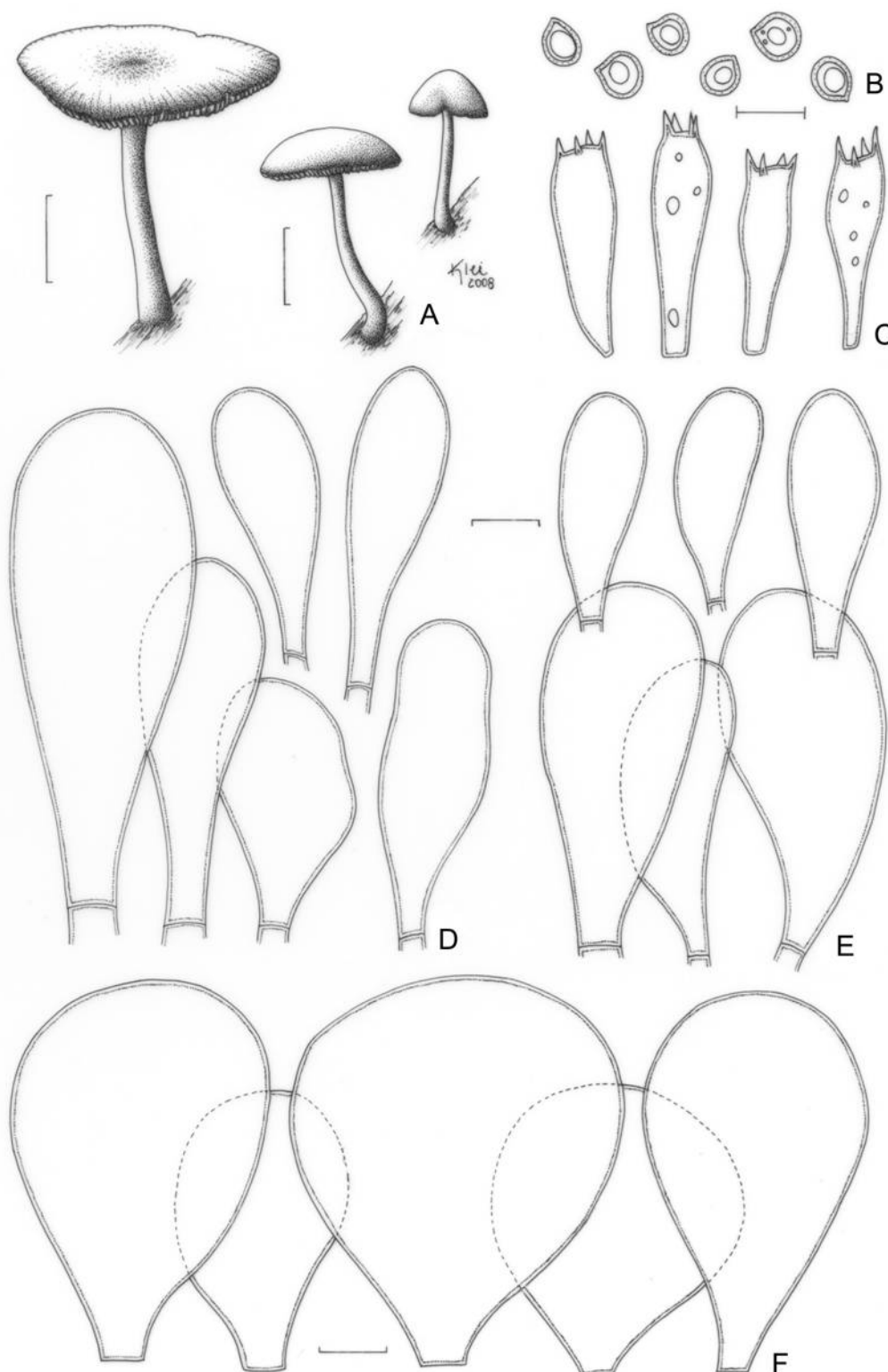


Fig 12 – *Pluteus sublaevigatus* (F. Karstedt et al. 1085). (A) Basidioma. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia. (E) Cheilocystidia. (F) Pileipellis cells. Bars (A) = 1 cm; (B–F) = 10 μ m.

walled, 4-spored. *Pleurocystidia* (34–)36–50(–65) \times 12.5–17.5(–25) μ m, clavate to vesiculose, sometimes slightly ventricose, very hyaline and difficult to see in KOH, sparse and very rare, thin-walled. *Cheilocystidia* (22–)31–49(–54) \times (7.5–)10–21(–26) μ m, like the

pleurocystidia, clavate to vesiculose, very hyaline, abundant, thin-walled. *Lamella edge* sterile. *Lamella trama* bilateral convergent, up to 62 μm width, composed of thin or slightly thick-walled hyphae, 3.7–10 μm diam., hyaline, septate. *Pileus context* undifferentiated, up to 187 μm thick, composed of thin or slightly thick-walled hyphae, 2.5–6.2(–16.2) μm diam., hyaline, septate. *Pileipellis* cellular up to 50 μm thick, composed of one layer of clavate, vesiculose or subglobose cells, (35–)44–51(–55) \times (21–)29–37 μm , with a short pedicel (2.5–7.5 μm long), thin-walled, hyaline. *Clamp connections* absent in all parts examined.

Habitat and substrate. Gregarious on decaying wood.

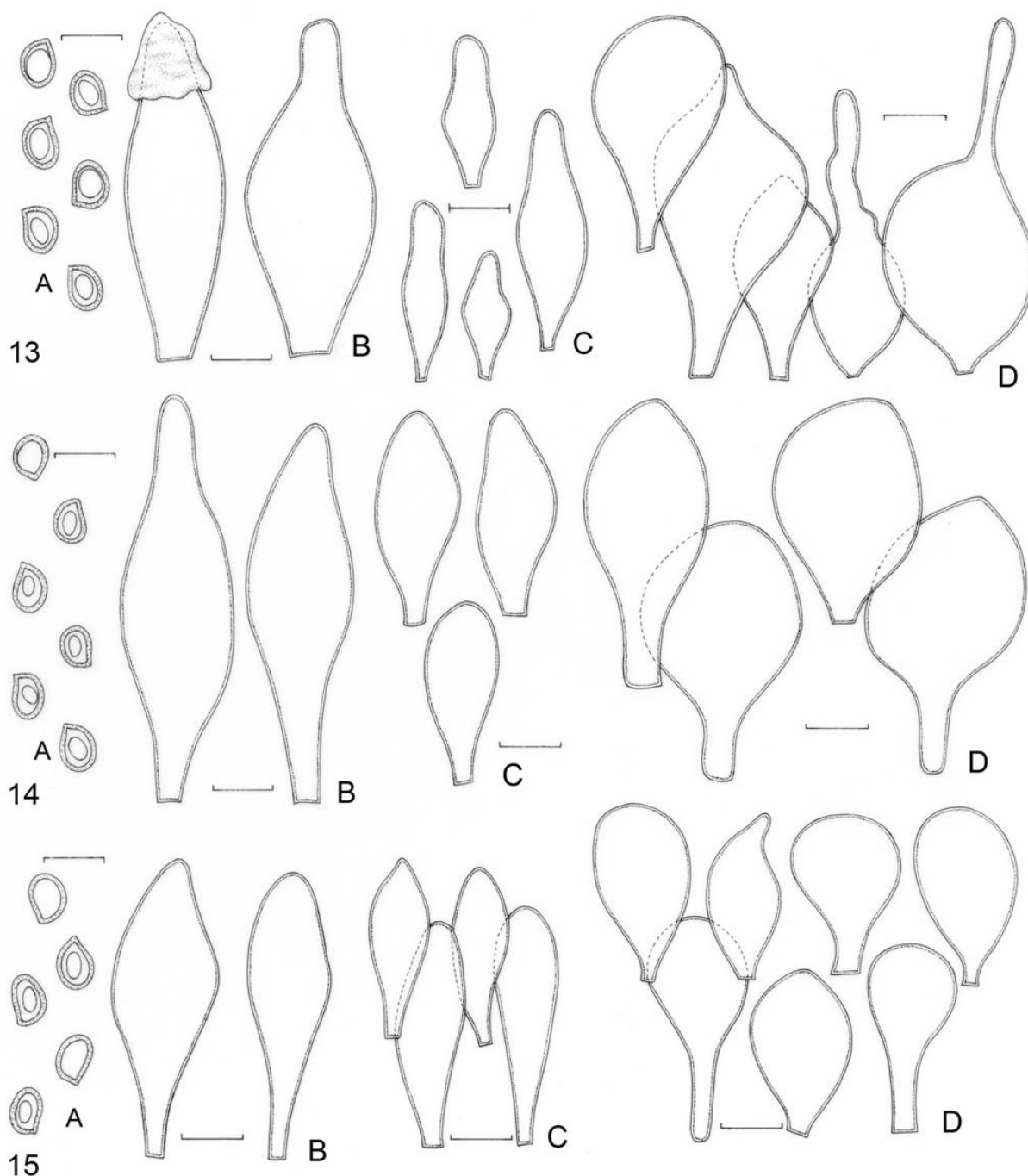
Specimens examined. **Bolivia:** Dpto La Paz, Prov. Nor-Yungas: Charobamba, 13 Feb. 1956, R. Singer 1147 (LIL—holotype). **Brazil:** São Paulo State: São Paulo City, Parque Estadual da Cantareira, Núcleo Engordador, 19 Feb. 2008, F. Karstedt et al. 1085 (SP).

Comments. The frequently globose basidiospores and the large subglobose cells of the pileipellis, together with the results of the molecular analyses are the base to propose the new status for *P. chrysophlebius* subsp. *sublaevigatus*.

Singer (1958) used the frequently globose basidiospores as the distinctive character to propose *P. chrysophlebius* subsp. *sublaevigatus* based on material collected in Bolivia. The Brazilian collection is slightly different because it has a pileus slightly rugose at the centre and pleurocystidia predominantly clavate to vesiculose and slightly longer than those described for *P. chrysophlebius* subsp. *sublaevigatus* that has pileus with “the disc seemingly smooth when wet, but in dry or dried condition becoming rugulose”, and cystidia “about 35 \times 15 μm if vesiculose, about 53 \times 9.5 μm if ampullaceous” (Singer 1958). These are minor differences that we consider intraspecific variations.

Pluteus sublaevigatus is close to *P. chrysophlebius* and *P. admirabilis* in morphology, but the molecular analysis of the nLSU gene (Fig 1) showed that they clustered in separated clades, with *P. chrysophlebius* and *P. admirabilis* in one clade and *P. sublaevigatus* in another clade with a Brazilian yellow species *P. aureovenatus*.

The morphological re-examination of the three collections, *P. admirabilis* (DAOM193532, DAOM197226) and *P. chrysophlebius* (DAOM190194) used in the molecular analyses showed some differences in microcharacters, especially in pileipellis cells (Fig 13–15). The pileipellis cells from these collections are usually clavate-vesiculose to subglobose, ranging in size and in the presence or absence of apical projections (Table 2). Moreover, the size and shape of basidiospores are a constant character; pleurocystidia are constantly slightly ventricose with few differences in size; and the cheilocystidia are frequently clavate-vesiculose to slightly ventricose (Table 2). These microcharacters partly agree with the type studies results presented by Banerjee & Sundberg (1993) and Singer (1956) for *P. admirabilis*



Figs 13–15 – (13) *Pluteus admirabilis* (DAOM193532). (14) *P. admirabilis* (DAOM197226). (15) *P. chrysophlebius* (DAOM190194). (A) Basidiospores. (B) Basidia. (C) Pleurocystidia. (D) Cheilocystidia. (E) Pileipellis cells. Bars = 10 µm.

and *P. chrysophlebius*, and they differ from those observed in the Brazilian collection of *P. sublaevigatus* (Table 2).

These differences are probably due to intraspecific variations, since in our analysis the nLSU sequences of *P. chrysophlebius* and *P. admirabilis* from GenBank clustered in a clade with 99% BS. This relationship between the sequences was already reported by Moncalvo *et al.* (2002) and Minnis *et al.* (2006). Then, if these collections really are representatives of

P. chrysophlebius and *P. admirabilis*, according to the morphological and molecular data presented, they cannot be considered independent species. This fact is in accordance with Murrill (1917) and Singer (1956) who already considered them as synonyms.

The study of the voucher collections from the GenBank sequences was important to establish the morphological differences between them and the Brazilian collections, and to support the results obtained with molecular analysis. *Pluteus sublaevigatus* differs from the vouchers by the close, not remote lamellae, basidiospores frequently globose, larger pileipellis cells, similar pleuro- and cheilocystidia and predominantly clavate-vesiculose (Table 2).

Table 2 – Micromorphological comparison between collections of *Pluteus sublaevigatus*, *P. admirabilis*, and *P. chrysophlebius*

Characters	Species / Collection			
	<i>P. admirabilis</i> DAOM193532	<i>P. admirabilis</i> DAOM197226	<i>P. chrysophlebius</i> DAOM190194	<i>P. sublaevigatus</i> <i>F. Karstedt et al. 1085</i>
Basidiospores	7.5 × 6.2 µm broadly ellipsoid	7.5 × 6.2 µm broadly ellipsoid	7.5 × 6.2 µm broadly ellipsoid	6.2–7.5 × 5–6.2 preponderantly globose
Pleurocystidia	47–71 × 16.2–21 µm slightly ventricose, with apical mucilage	50–81 × 17.5–25 µm slightly ventricose	41–52 × 10–18.7 µm slightly ventricose	36–50 × 12.5–17.5 µm clavate to vesiculose
Cheilocystidia	21–38 × 7.5–12.5 µm clavate-vesiculose to slightly ventricose	26.2–50 × 10–18.7 µm clavate-vesiculose to slightly ventricose	30–40 × 10–11.2 µm clavate-vesiculose to slightly ventricose	31–49 × 10–21 µm clavate to vesiculose
Pileipellis cells	16.2–51 × 12.5–25 µm with or without a medium pedicel up to 12.5 µm long, frequently with a short to long apical projection approximately 5–25 µm long	35–49 × 13.7–25 µm with a short to medium pedicel up to 13.7 µm long, without apical projections, and sometimes with a broadly obtuse apex	26–37.5 × 12.5–17.5 µm with a short to medium pedicel approximately 2.5–15 µm long, and rarely subventricose with a broadly obtuse apex up to 5 µm long	44–51 × 29–34 µm with a short pedicel approximately 2.5–7.5 µm long

Stijve & Meijer (1993) and Meijer (2006) recorded in a checklist the occurrence of *P. chrysophlebius* ssp. *bruchii* Singer for Paraná State, Brazil, that probably represents *P. sublaevigatus*. The record of *P. chrysophlebius* for Martinique and Guadeloupe by Pegler (1983), also probably represents *P. sublaevigatus* due to the description and illustrations presented. *Pluteus sublaevigatus* belongs to section *Celluloderma*, subsection *Eucellulodermini*.

Pluteus xylophilus (Speg.) Singer, *Lilloa* **23**: 203 (1951). (Fig 16)

Pileus 30–70(–110) mm diam., plane-convex, occasionally slightly concave, sometimes slightly umbonate, light brown (N₆₀A₆₀M₅₀ to N₆₀A₄₀M₄₀), darker at the centre (N₈₀A₅₀M₅₀), paler and discolouring radially towards the margin, margin sometimes splitting and apparently

not sulcate or striate. *Lamellae* remote, slightly pinkish to pinkish, crowded, with lamellulae. *Stipe* 35–60(–120) × 3–10(apex)–9–20(base) mm, tapering towards the apex, rarely equal with 3 mm diam., central, sometimes flexuous, cream with brown fibrils over the surface and especially at base, longitudinally striate, with scanty mycelium at base. *Basidiospores* [160/8/6] (5–)6.2–7.5(–8.7) × 3.7–6.2 μm (Q = 1.2–1.6), broadly ellipsoid to ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. *Basidia* (18.7–)21–26 × 6.2–8.7 μm, clavate or versiform, thin-walled, 4-spored, with small scattered guttulate. *Pleurocystidia* of three types: (I) the normal *Cervinus*-type, (46–)52–75(–87) × 12.5–23(–25) μm, fusoid-ventricose, hyaline, apices usually with 2–6 lateral prongs, moderately thick-walled especially in the apical region and tapering towards the base, sparse, and abundant to very abundant, frequently with adhered basidiospores on many pleurocystidial apices; (II) the modified *Cervinus*-type as found in *P. harrisii*, usually without prongs and subcapitate or slightly strangulated at the apex, or sometimes with two short lateral prongs, size similar to type I; (III) preponderantly of the *Magnus*-type, usually shorter especially in length, 38–62 × (8.7–)11–28 μm, lageniform to fusoid, with acute apex or rarely with a rounded apex, hyaline, thin to moderately thick-walled, rare. *Cheilocystidia* dimorphic, not abundant, very rooting on hymenium, and hard to see: (I) short vesiculose, clavate to subclavate, 21–37 × 8.7–15 μm; (II) long-cylindrical-clavate, sometimes with a moderately long pedicel, 40–69 × 10–15 μm; sometimes it is found transitions between the two extreme forms which are usually slightly ventricose or versiform and moderately deformed. *Lamella edge* heteromorphous. *Lamella trama* bilateral convergent, up to 62 μm width, composed of thin or slightly thick-walled hyphae, sometimes moderately inflated, 1.2–13.7 μm diam., hyaline, septate. Pileus context undifferentiated, approximately 187 μm thick, occasionally very thick more than 1,000 μm thick, composed of slightly thick-walled hyphae, sometimes inflated, 3.7–21 μm diam., hyaline, septate. Pileipellis a repent epicutis up to 125 μm thick, composed of thin to slightly thick-walled hyphae, 3.7–6.2 μm diam., elongated, septate, with dissolved brown vacuolar content. *Clamp connections* absent in all parts examined.

Habitat and substrate. Solitary to scattered on wood.

Specimens examined. **Brazil:** São Paulo State: São Paulo City, Parque Estadual das Fontes do Ipiranga, 10 Nov. 1982, G. Guzmán 22986 (SP); 16 Jan. 1987, Pegler et al. 3712 (SP); 20 June 2006, F. Karstedt et al. 683 (SP); 30 May 2007, Menolli Jr. et al. 138 (SP); 18 Oct. 2007, Menolli Jr. & F. Karstedt 150 (SP); Parque Estadual da Cantareira, Núcleo Engordador, 21 Aug. 2007, Menolli Jr. et al. 143 (SP); 23 Oct. 2008, M. Capelari & L.A.S. Ramos 4397 (SP).

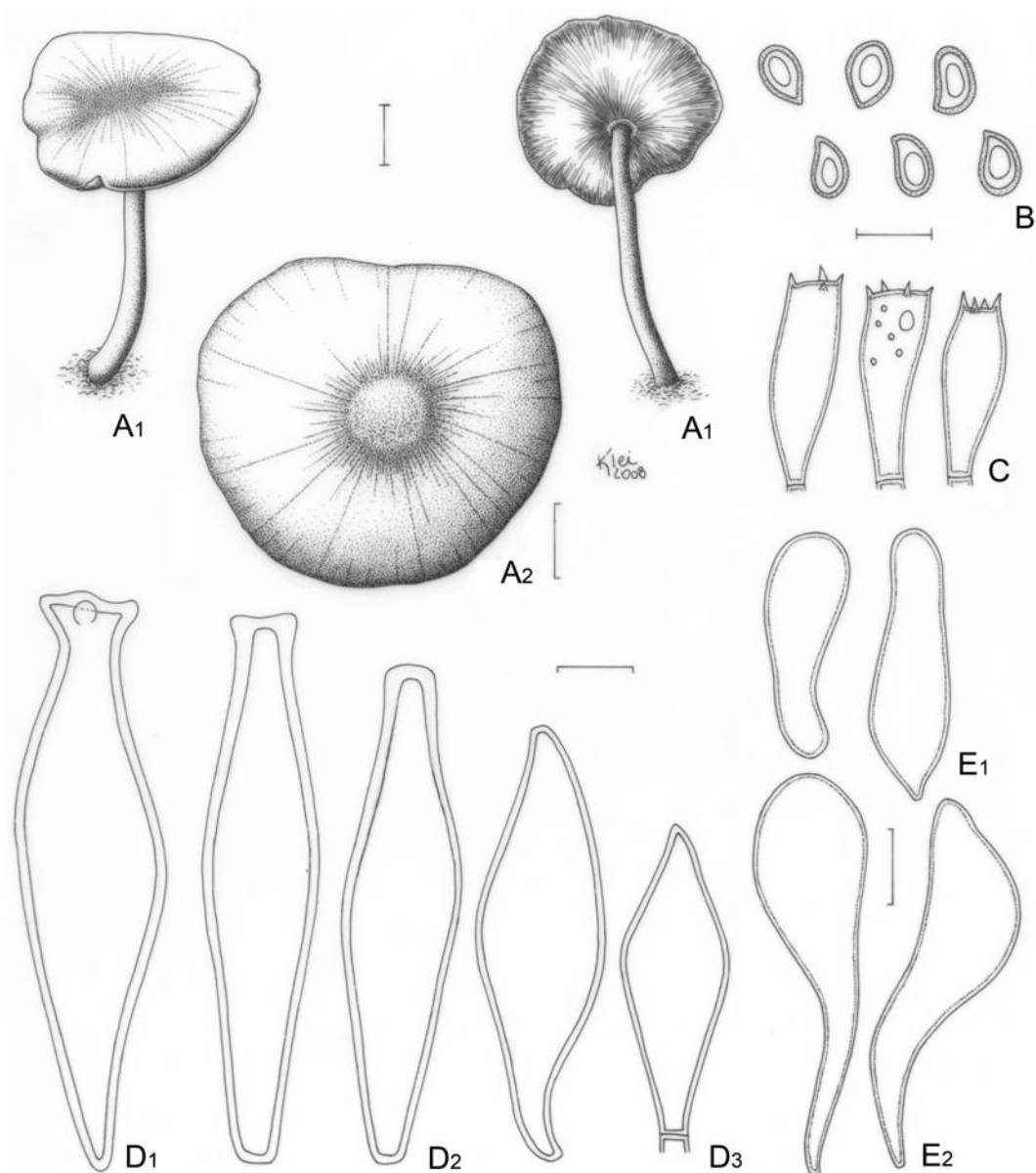


Fig 16 – *Pluteus xylophilus*. (A) Basidioma; A₁. Menolli Jr. et al. 138 ; A₂. Menolli Jr. et al. 143. (B–D) Menolli Jr. et al. 143. (B) Basidiospores. (C) Basidia. (D) Pleurocystidia; D₁. Normal *Cervinus*-type; D₂. Modified *Cervinus*-type; D₃. *Magnus*-type. (E) Cheilocystidia (Menolli Jr. et al. 138); E₁. Short vesiculose; E₂. Long-cylindrical-clavate. Bars (A) = 1 cm; (B–E) = 10 μ m.

Comments. *Pluteus xylophilus* is characterised by a moderately large pileus, three types of pleurocystidia, and two types of cheilocystidia. The specimens herein studied have the surface usually with some cavities, probably caused by insects, showing the white flesh.

Singer (1958) described two varieties, viz. *P. xylophilus* var. *tucumanensis* Singer and *P. xylophilus* var. *major* Singer, based on the size and pigmentation differences of the pileus and predominance of each cheilocystidia type. However, he emphasises that would be necessary to check whether each of these characters remains constant in successive fruitings and to verify if these morphological variations constitute forma, varieties, or independent

microspecies. Singer's varieties are not considered in this work because considerable morphological variations were not observed.

Singer (1956, 1958) accepted two varieties of *P. cervinus* P. Kumm., viz. *P. cervinus* var. *brasiliensis* and *P. cervinus* var. *tucumanensis* Singer, as synonyms of *P. xylophilus*; and *P. cervinus* sensu Rick (1938), which were recorded for Brazil, as a probable *P. xylophilus*.

The geographic distribution of *P. xylophilus* includes Argentina (Spegazzini 1925 as *Entoloma xylophilum*; Singer 1952 as *P. cervinus* var. *tucumanensis* and *P. cervinus* var. *brasiliensis*; Singer 1958), Bolivia (Singer 1958), Brazil (Bresadola 1920 as *P. cervinus* var. *brasiliensis*; Grandi *et al.* 1984; Stijve & Meijer 1993 as *P. xylophilus* and *P. xylophilus* var. *tucumanensis*; Pegler 1997; Meijer 2006), and Peru (Singer 1958). *Pluteus xylophilus* belongs to section *Pluteus*.

Acknowledgements

The authors thank Dr. Tarciso S. Filgueiras, Reserva Biológica do IBGE, and José Francisco Kuhar, FCEN Universidad de Buenos Aires, who kindly revised the Latin diagnoses; the curators of DAOM, F, K and LIL herbaria for the material loan; Dr. Maria Helena Pelegrinelli Fungaro, Universidade Estadual de Londrina, for some DNA sequencing; Fernanda Karstedt, Instituto de Botânica, for collecting some specimens; Klei R. Sousa for preparing the illustrations; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the support and grant to the first author; and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 04/04319-2) for financial support.

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CAPÍTULO IV

Records and two new species of *Volvarellia* (*Pluteaceae*, *Agaricales*) from Brazil*

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*Artigo publicado na revista *Mycotaxon* 106: 385–398. 2008.

MYCOTAXON

Volume 106, pp. 385–398

October–December 2008

**Records and two new species of *Volvariella*
(*Pluteaceae*, *Agaricales*) from Brazil**

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Abstract — Two new species of *Volvariella*, *V. heterospora* and *V. nullicystidiata*, are described and illustrated from specimens collected at the Parque Estadual da Cantareira, São Paulo, SP, Brazil. *V. heterospora* is characterized by its two types of basidiospores, whereas *V. nullicystidiata* lacks cystidia. *Volvariella bombycina* and *V. perniciosa*, both collected in the same area, are also described, illustrated and recorded. The latter represents the first record for São Paulo State and the second record for Brazil. In addition, the specimens of *Volvariella* deposited at herbaria SP and collected in remnants of the Atlantic forest of the metropolitan region of São Paulo, SP, Brazil, were re-evaluated.

Key words — *Basidiomycota*, diversity, taxonomy

Introduction

Volvariella Speg. is a widely distributed genus whose species have been reported from tropical, subtropical, and temperate regions of both eastern and western hemispheres (Shaffer 1957). The genus is characterized by bulbangiocarpic development with a membranous or fleshy volva, annulus absent, free and pinkish lamellae, inamyloid basidiospores with a stramineous thickened wall, and bilateral convergent hymenophoral trama (Pegler 1983).

The genus comprises ca. 50 species (Kirk et al. 2001) with 17 mentioned for Brazil. Montagne (1856) was the first author to record species of *Volvariella* from Brazil, with *V. cnemidophora* (Mont.) Singer (as *Agaricus cnemidophorus*). Rick (1930, 1961) recorded eight species of *Volvariella* for Rio Grande do Sul State, viz. *V. bombycina* (as *Volvaria bombycina*), *V. cnemidophora* (as *Volvaria cnemidophora*), *V. fibrillosa* (Bres. ex Rick) Singer (as *Volvaria fibrillosa*), *V. pusilla* (Pers.) Singer (as *Volvaria parvula*), *V. surrecta* (Knapp) Singer (as *Volvaria loveana*), *V. taylorii* (Berk. & Broome) Singer (as *Volvaria taylorii*),

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V. thwaitesii (Hook. f.) G.C. Rath (as *Volvaria thwaitesii*), and *V. volvacea* (Bull.) Singer (as *Volvaria volvacea*).

For Pernambuco State, Batista (1957) described *V. oswaldoi* (Bat.) Putzke (as *Volvaria oswaldoi*) and recorded *V. gloiocephala* (DC.) Boekhout & Enderle (as *Volvaria speciosa*), and later Batista & Bezerra (1960) also mentioned *V. cnemidophora*. Singer (1989) described two new species, *V. macrospora* Singer and *V. rondoniensis* Singer, from Amazonas and Rondônia States respectively. For São Paulo State, *V. bakeri* (Murrill) Shaffer (Grandi et al. 1984, Pegler 1997), *V. bombycina* (Bononi et al. 1981) and *V. taylorii* (Pegler 1997) were mentioned. For Paraná State, ten species of *Volvariella* were mentioned by Meijer (2001, 2006), including *V. bombycina*, *V. earlei* (Murrill) Shaffer, *V. gloiocephala*, *V. hypopithys* (Fr.) M.M. Moser, *V. cf. murinella* (Quél.) M.M. Moser, *V. perciliata*, *V. cf. pusilla* var. *taylorii* (Berk. & Broome) Boekhout, *V. rondoniensis*, *V. cf. striata* N.C. Pathak and *V. volvacea*.

Beside these species, there is the record of three species of *Volvaria* (Fr.) P. Kumm. for Rio Grande do Sul State that were not yet combined in *Volvariella*, viz. *Volvaria pubipes* (Peck) Sacc. (Rick 1938, 1961), *Volvaria rhodomelas* (Lasch) P. Kumm. (Rick 1930, 1938, 1961) and *Volvaria umbonata* var. *brasiliensis* Rick (Rick 1930).

In this article, we report on four species of *Volvariella* recently collected at the Parque Estadual da Cantareira, São Paulo, SP, Brazil, two of which are described as new. In addition, the specimens deposited as *Volvariella* at the Herbário do Estado Maria Eneyda P. Kauffmann Fidalgo (SP) and collected in remnants of Atlantic forest of metropolitan region of São Paulo City, SP, Brazil were also re-evaluated.

Material and methods

The specimens were collected at the Parque Estadual da Cantareira, a remnant of Atlantic forest in São Paulo State, Brazil and deposited at herbarium SP. The macroscopical descriptions and illustrations of basidiomata were based on fresh material. Color terms are from Küppers (1979). The material deposited as *Volvariella* at herbaria SP was revised from herbarium material originally collected at the Parque Estadual das Fontes do Ipiranga (PEFI), a remnant of Atlantic forest inside of urban area of São Paulo City, SP, Brazil. For microscopic analysis, the dried material was rehydrated in 70% ethanol followed by 5% KOH or Melzer's reagent. All microscopic illustrations were made with the aid of a drawing tube. The notation "[a/b/c]" at the beginning of a set of spore data is to be read, "'a' spores were measured from 'b' basidiomata taken from 'c' collections", and the Q represents the range of length/width quotients for all spores measured. The SEM images followed Baroni (1981).

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DNA sequences of the large subunit (LSU) and internal transcribed space (ITS) region of nuclear ribosomal DNA were obtained from three species, for future phylogenetic molecular studies. GenBank accession numbers are cited below the species name at the beginning of the formal description.

Results

Three collections deposited as *Volvariella* at SP and collected in Atlantic forest remnants in the metropolitan area of São Paulo City were re-evaluated. Two collections (SP47345 and SP47623) previously identified as *V. bombycina* were destroyed by insects and moulds so that it was impossible to confirm their identifications. Re-examination of the third collection labeled as *V. bakeri* (SP128034) demonstrated that it represents a species of *Pluteus* Fr. section *Pluteus* as indicated by the absence of a volva in the basidioma and presence of horned metuloid cystidia, which is typical of section *Pluteus*. This result invalidated the record of *V. bakeri* for Brazil as mentioned by Grandi et al. (1984) and Pegler (1997).

The other specimens studied in this work collected at the Parque Estadual da Cantareira are described below.

Volvariella bombycina (Schaeff.) Singer
GENBANK EU920669, EU920673

FIGS. 1, 5A-B

MACROCHARACTERS — PILEUS large, 140 mm diam., campanulate to convex, slightly umbonate, whitish-cream, surface dry, smooth at center with yellowish fibrils toward the margin. LAMELLAE remote, pinkish, crowded, with lamellulae. STIPE 145 × 11(apex)–17(base) mm, yellowish-cream, tapering toward the apex and slightly bulbous, flexuous, slightly fibrillose at the apex and slightly longitudinally striate toward the base. VOLVA 40 mm long, saccate, yellowish-white, ample, well developed, often deliquescent, with scanty mycelium at the base.

MICROCHARACTERS — BASIDIOSPORES [20/1/1] 8.7(–10) × 6.2(–7.5) μm (Q = 1.33–1.4), ellipsoid, smooth, slightly pinkish, inamyloid, thick-walled, guttulate, often 2-guttulate. BASIDIA (18.7–)20–23(–25) × (6.2–)7.5–8.7 μm, clavate, thin-walled, with small guttulae, 4-spored, with short sterigmata. PLEUROCYSTIDIA (36–)40–64(–70) × (7.5–)8.7–15(–17.5) μm, appendiculate or ventricose, sometimes strangulate at the apex or throughout extension, fairly abundant and well distributed, thin-walled. CHEILOCYSTIDIA (50–)60–94(–100) × (13.7–)16.2–24 μm, clavate to slightly fusoid, very abundant, thin-walled. LAMELLA TRAMA bilateral convergent, 50–89 μm width, of thin-walled hyphae, 3.7–11.2 μm diam., hyaline, sometimes pale yellowish, septate. SUBHYMENIUM cellular. PILEUS CONTEXT undifferentiated, composed by thin-

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FIGURE 1: *Volvariella bombycina*. a. Basidioma (Scale bar = 1 cm).
b. Basidiospores. c. Basidia. d. Pleurocystidia. e. Cheilocystidia. Scale bars = 10 μ m.

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walled hyphae, 6.2–12.5 μm diam., hyaline, septate. PILEIPPELLIS composed by thin-walled hyphae, 5–18 μm diam., with yellowish content, arranged more or less parallel to surface. VOLVAL ELEMENTS formed by thin-walled hyphae, 3.7–13.7 μm diam., hyaline or with light brown content, septate, well compacted and parallel. CLAMP CONNECTIONS absent in all parts examined.

HABITAT AND SUBSTRATE — Solitary on decaying wood.

SPECIMENS EXAMINED — BRAZIL. SÃO PAULO STATE: São Paulo, PARQUE ESTADUAL DA CANTAREIRA, NÚCLEO ENGORDADOR — 30.X.2007, *Menolli Jr. et al.* 151 (SP).

COMMENTS — *Volvariella bombycina* is a widely distributed species and recognized by its robust habit, pale silky-scaly pileus, well-developed saccate volva, habitat on wood, and medium-sized basidiospores. Previous reports of *V. bombycina* from Brazil include Rick (1961) for Rio Grande do Sul State, and in the checklists of Bononi et al. (1981) and Meijer (2001, 2006) from São Paulo and Paraná States respectively.

***Volvariella heterospora* Menolli & Capelari, sp. nov.**

FIGS. 2, 5C–D

MYCOBANK MB 512149

Pileus 43 mm *latus*, *conicus-convexus*, *umbonatus*, *atrocinereus*, *fibrillosus*. *Lamellae liberae*, *roseae*. *Stipes* 68 mm *altus*, *apice usque ad 4 mm latus*, *basi 8 mm latis*, *albidus*. *Volva* 170 \times 8 mm, *adnata vel limbata*, *alba*, *externa coacta*. *Basidiosporae dimorphae*: *typus I* (6.4–)7.2(–8) \times (4–)4.8(–5.6) μm , *late ellipsoideae vel ellipsoideae*, *raro elongatus*; *typus II* (8.8–)9.6–12(–13.6) \times (4.8–)5.6–6.4 μm , *elongatus*, *raro ellipsoideae*, *plerumque deformans*. *Basidia* (16.2–)20–21 \times 6.2–8.7 μm , *clavate*, *4-sporigera*. *Pleurocystidia* (32–)37–49 \times (6.2–)7.5–10(–11.2) μm , *versiformis*, *appendiculate*, *ventricosa vel lageniformis*, *numerosa*. *Cheilocystidia* (26–)34–41(–49) \times (5–)7.5–10(–11.2) μm , *clavata vel ventricosa*. *Trama himenophoralis inverse bilateralis*, *hyaline*. *Cuticula pilei trichodermate*, *cellulis terminalis elongates*, *inflates*, *erectis vel suberectis*. *Hyphae omnes defibulatae*.

TYPE SPECIMENS — BRAZIL. SÃO PAULO STATE: São Paulo, PARQUE ESTADUAL DA CANTAREIRA, NÚCLEO ENGORDADOR — 12.XII.2006, *F. Karstedt & M. Capelari* 832 (HOLOTYPE-SP).

ETYMOLOGY: the name refers to the different types of basidiospores.

MACROCHARACTERS — PILEUS medium-sized, 43 mm diam., conico-convex, umbonate, dark gray with slightly darker center, surface dry, covered by radially arranged gray fibrils, margin entire, sometimes exposing the lamellae. LAMELLAE free to remote, pinkish, crowded, with lamellulae. STIPE 68 \times 4(apex)–8(base) mm, white, tapering towards the apex, slightly subbulbose, surface somewhat pruinose. VOLVA 170 \times 8 mm, adnate to limbate, white, well adhered to the stipe, enclosing it like a sock, with a cleavage line at one side of stipe and at another side forming an adhered extension like a limb above the base, outer surface slightly felted.

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MICROCHARACTERS — BASIDIOSPORES [60/1/1] (6.4–)7.2(–8) × (4–)4.8(–5.6) μm (Q = 1.28–1.8), broadly ellipsoid to ellipsoid, rarely elongate, or bigger (8.8–)9.6–12(–13.6) × (4.8–)5.6–6.4 (Q = 1.5–2.43), elongate, rarely ellipsoid, frequently deformed and inequilateral or with the proximal end more tapering than the distal end, smooth, hyaline to slightly pinkish, inamyloid, thick-walled, guttulate, sometimes 2-guttulate, rarely 3-guttulate or nonguttulate. BASIDIA (16.2–)20–21 × 6.2–8.7 μm , clavate, thin-walled, with small scattered guttulae, 4-spored. PLEUROCYSTIDIA (32–)37–49 × (6.2–)7.5–10(–11.2) μm , versiform, appendiculate or ventricose, sometimes lageniform 54–62 × 20–22 μm , abundant and sparse, very hyaline and hard to see in KOH, thin-walled. CHEILOCYSTIDIA (26–)34–41(–49) × (5–)7.5–10(–11.2) μm , clavate to ventricose, not abundant, hyaline and also difficult to see in KOH, thin-walled. LAMELLA EDGE heteromorphous. LAMELLA TRAMA bilateral convergent, 44–50 μm width, of thin or slightly thick-walled hyphae, 2.5–17.5 μm diam., hyaline, septate. SUBHYMENIUM cellular. PILEUS CONTEXT undifferentiated, 31–44 μm thick, composed by thin-walled hyphae, 3.7–18.7 μm diam., hyaline, septate. PILEPELLIS trichodermic, thick, up to 640 μm thick, composed by thin or slightly thick-walled hyphae, 5–26 μm diam., elongated, with terminal elements slightly inflated, septate, with light brown content, arrangement erect or sub-erect. VOLVAL ELEMENTS formed by thin-walled hyphae, 3.7–7.5 μm diam., hyaline, frequently not septate, interwoven. CLAMP CONNECTIONS absent in all parts examined.

HABITAT AND SUBSTRATE — Solitary on litter.

COMMENTS — *Volvariella heterospora* is characterized by a small- to medium-sized pileus that is conic-convex, umbonate, and gray with a slightly darker center and by two types of basidiospores, one short, broadly ellipsoid to ellipsoid, and the other bigger, elongate, rarely ellipsoid, and frequently deformed.

This species is similar to *V. murinella*, especially in macroscopic characteristics (Boekhout 1986). But according to this author, *V. murinella* has a low broad umbo, longer basidia [20–30(–35) × 7–10 μm], much longer cystidia [40–100 × 10–30(–50)], uniform basidiospores not exceeding 8.3 μm in length and 4.4 μm in width [or 5 μm according to Orton (1986)]. There is no mention of the two basidiospore types or of the presence of bigger and frequently deformed basidiospores, or of a volval extension along one side of the stipe. Additionally, *V. heterospora* differs in pileus color and shape when compared to the description of *V. murinella* by Lange (1935), who described it as pale ashy gray and convex; Lange's illustration, which was also selected by Boekhout (1986), represents a specimen with totally convex pileus, without evident umbo or conic shape.

Volvariella spp. nov. (Brazil) ... 391

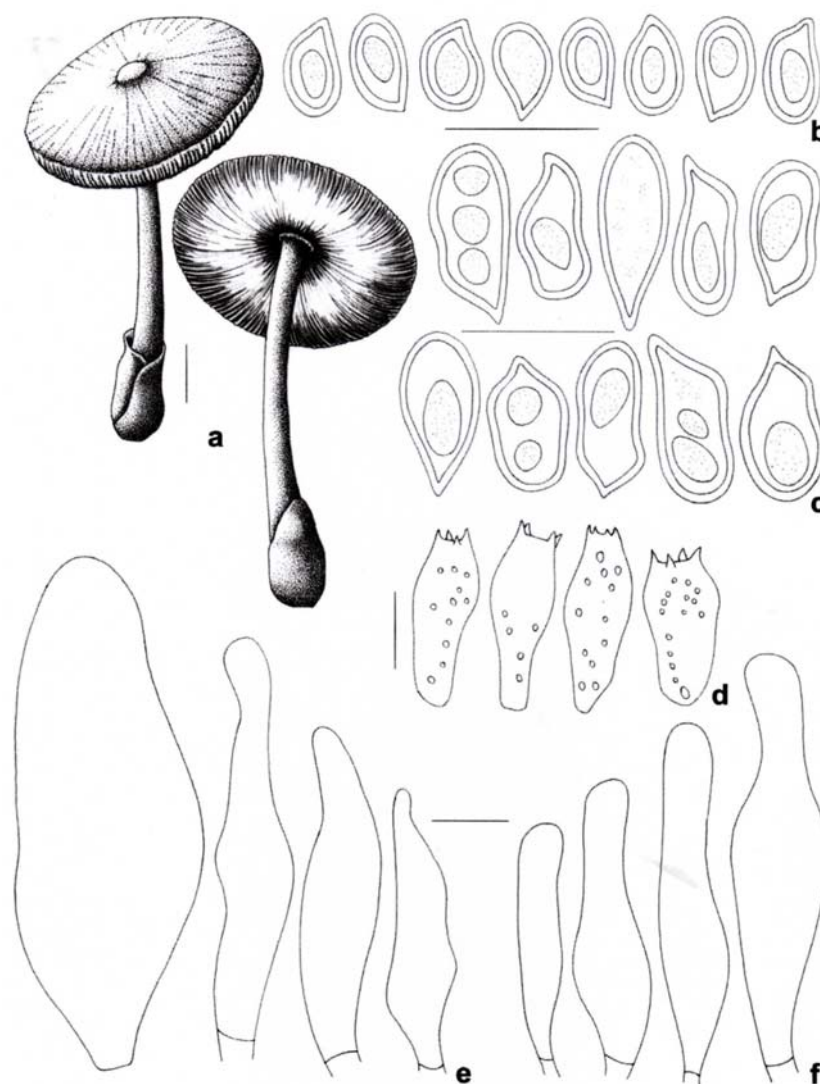


FIGURE 2: *Volvariella heterospora* (holotype). a. Basidioma (Scale bar = 1 cm).
 b. Small basidiospores. c. Large basidiospores. d. Basidia. e. Pleurocystidia. f. Cheilocystidia.
 Scale bars = 10 µm.

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Volvariella heterospora also resembles *V. taylorii*, but the latter is differentiated by a gray, gray-olivaceous, hazel, brown, grayish avellaneous or grayish date-brown volva (Lange 1935, Shaffer 1957, Pegler 1983, Orton 1986).

***Volvariella nullicystidiata* Menolli & Capelari, sp. nov.**

FIGS. 3, 5E-F

MYCOBANK MB 512150; GENBANK EU920670, EU920671

Pileus 66 mm *latus*, *convexus*, *umbonatus*, *brunneus*, *pallidus cinereus ad marginem*. *Lamellae liberae*, *roseae*. *Stipes* 108 mm *altus*, *albidus*, *apice usque ad 4.4 mm latus*, *basi usque ad 8 mm latis*. *Volva* 28 × 200 mm, *saccata*, *trilobata*, *cupuliformis*, *cremeus*, *cum mycelium basali*. *Basidiosporae* (7.5–)8.7 × (5–)6.2(–7.5) μm, *ellipsoideae*, *raro late ellipsoideae vel ovoideae*. *Basidia* (22–)25–26(–28) × (7.5–)8.7–10(–11.2) μm, *clavata*, *4-sporigera*. *Pleurocystidia et cheilocystidia absentibus*. *Trama hymenophoralis inverse bilateralis*, *hyalina*. *Cuticula pilei hyphis repentibus*. *Hyphae omnes defibulatae*.

TYPE SPECIMENS — BRAZIL. SÃO PAULO STATE: São Paulo, PARQUE ESTADUAL DA CANTAREIRA, NÚCLEO ENGORDADOR — 31.I.2008, F. Karstedt *et al.* 1055 (HOLOTYPE-SP).

ETYMOLOGY: the name refers to the total absence of cystidia.

MACROCHARACTERS — PILEUS medium-sized, 66 mm diam., convex umbonate, dark brown at center, discoloring radially towards the margin, margin light gray, surface dry, covered by radially arranged brown-grayish fibrils, margin entire, slightly sulcate. LAMELLAE free, pinkish, with lamellulae. STIPE 108 × 4.4(apex)–8(base) mm, white-cream, light sordid grayish on base, tapering towards the apex, flexuous, slightly longitudinally striate. VOLVA 28 × 20 mm, saccate, beige, cupuliform, firm, well developed, 3-lobate, with cottony mycelium at the base.

MICROCHARACTERS — BASIDIOSPORES [20/1/1] (7.5–)8.7 × (5–)6.2(–7.5) μm (Q = 1.16–1.5), ellipsoid rarely broadly ellipsoid, with the distal end wider and broader than the proximal end (egg-shaped), smooth, slightly pinkish, inamyloid, thick-walled, guttulate, with a small apiculus hard to note and seemingly truncate. BASIDIA (22–)25–26(–28) × (7.5–)8.7–10(–11.2) μm, clavate, thin-walled, sometimes with small guttulae, 4-spored. PLEUROCYSTIDIA and CHEILOCYSTIDIA absent. LAMELLA EDGE fertile. LAMELLA TRAMA bilateral convergent, up to 31 μm width, of thin or slightly thick-walled hyphae, 3.7–15 μm diam., hyaline, septate. PILEUS CONTEXT undifferentiated, approximately 94 μm thick, composed by slightly thick-walled hyphae, 3.7–12.5 μm diam., hyaline, septate. PILEIPELLIS up to 225 μm thick, composed by thin-walled hyphae, 10–30 μm diam., with light brown content, septate, arrangement more or less parallel to the surface. VOLVAL ELEMENTS composed of thin or slightly thick-walled hyphae, 2.5–15(–27) μm diam., some inflated, hyaline, septate, interwoven. CLAMP CONNECTIONS absent in all parts examined.

HABITAT AND SUBSTRATE — Solitary on soil.

Volvariella spp. nov. (Brazil) ... 393

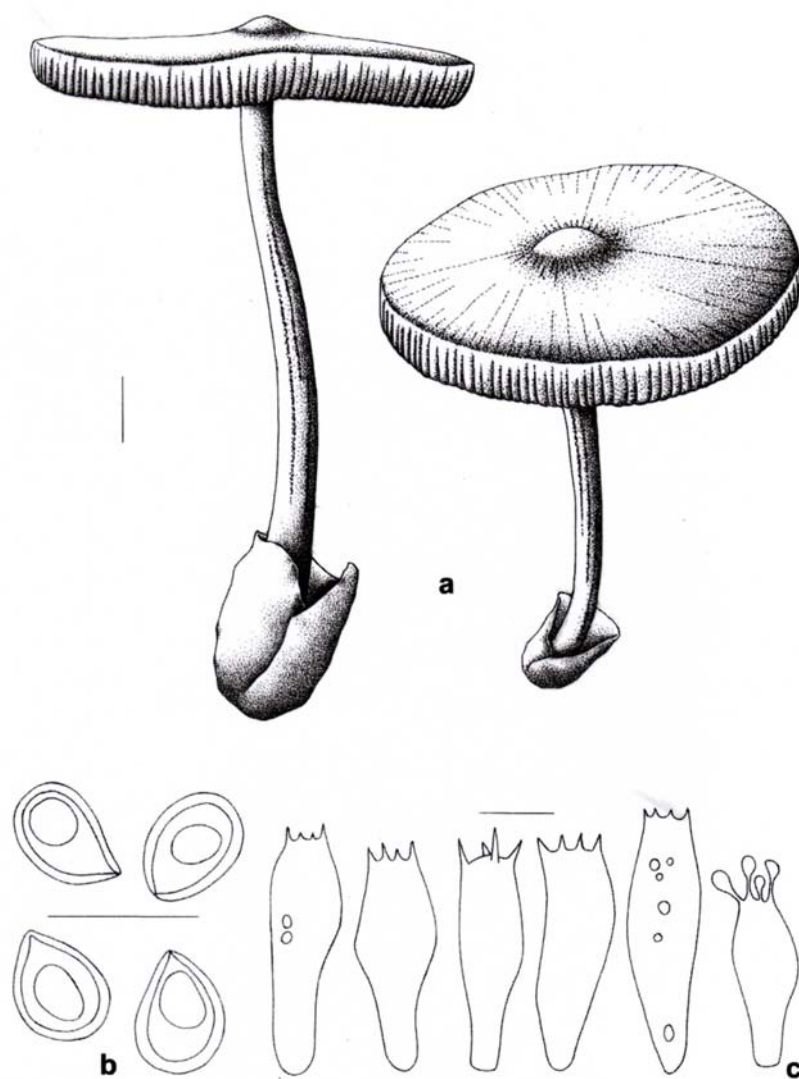


FIGURE 3: *Volvariella nullicystidiata* (holotype). a. basidioma (scale bar = 1 cm).
b. basidiospores. c. basidia. scale bars = 10 μ m.

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COMMENTS — *Volvariella nullicystidiata* is characterized by the complete absence of cystidia. Few species of *Volvariella* lack cystidia, among them are *V. acystidiata* N.C. Pathak and *V. jamaicensis* (Murrill) Shaffer. *Volvariella acystidiata* is quite different from *V. nullicystidiata* because it has basidiospores that are much longer [$14.2\text{--}17.1\text{--}(19.9) \times 8.5\text{--}10$] as described in the protologue (Pathak 1975). In addition, *V. acystidiata* has a narrower (25 mm) cream-colored viscid pileus, white and fragile volva, and basidia that are slightly longer ($26.4\text{--}37.2 \times 14.4\text{--}15.6 \mu\text{m}$) as described by Mora (1985) from material collected in Mexico. However, *V. nullicystidiata* is closely related to *V. jamaicensis*. The latter differs by the gregarious habit, slightly narrower pileus (50 mm) with slightly granular surface, shorter stipe ($50 \times 3\text{--}5$ mm), and a rather delicate, narrow, avellaneous and slightly shorter (10–20 mm) volva as described in the protologue (Murrill 1911). Besides, *V. jamaicensis* has shorter and much narrower basidiospores, which were cited in the protologue as $5 \times 3 \mu\text{m}$ and in the type study by Shaffer (1957) as $6.2\text{--}8 \times 3.3\text{--}4.3 \mu\text{m}$ with a larger Q value of 1.54–2.08.

Volvariella perciliata Courtec.

FIGS. 4, 5G–H

GENBANK EU920668, EU920671

MACROCHARACTERS — PILEUS small, 16–17 mm diam., convex with mammilate umbo, grayish-brown ($N_{70}A_{40}M_{30}$) with a darker center, discoloring radially towards the margin, surface dry, with short fibrils radially arranged, margin ciliate throughout its extension. LAMELLAE free, pinkish, with lamellulae. STIPE 30–50 \times 1.4–2 mm, white, cylindric, slightly pruinose, mostly on apical half, covered by many humidity drops throughout its surface. VOLVA 5 \times 8 mm, saccate, white to slight brownish, firm, like an egg shell, 4-lobate, pubescent.

MICROCHARACTERS — BASIDIOSPORES [$40/2/2$] ($6.2\text{--}7.5\text{--}8.7\text{--}(10) \times (3.7\text{--}5\text{--}(6.2) \mu\text{m}$ (Q = 1.4–2), ellipsoid to elongate, smooth, inamyloid, thick-walled, guttulate, often 2-guttulate, with a small apiculus hard to note. BASIDIA (21–) 23–29(–31) \times ($6.2\text{--}7.5\text{--}8.7\text{--}(10) \mu\text{m}$, clavate, thin-walled, with small guttulae, 4-spored, with short sterigmata. PLEUROCYSTIDIA 47–65 \times 6.2–12.5 μm , fusiform occasionally slightly ventricose, sparse, thin-walled. CHEILOCYSTIDIA abundant, like the pleurocystidia. LAMELLA TRAMA bilateral convergent, 37–44 μm width, of thin-walled hyphae, 2.5–15 μm diam., hyaline, septate. SUBHYMENIUM cellular. PILEUS CONTEXT undifferentiated, approximately 125–187 μm thick, constituted by thin-walled hyphae, 3.7–13.7 μm diam., hyaline, septate. PILEIPELLIS trichodermic, up to 187 μm thick, composed by thin-walled hyphae, 6.2–15 μm diam., elongated with the sub-terminal elements inflated, septate, with light brown content, arrangement erect or sub-erect. VOLVAL ELEMENTS composed of slightly thick-walled hyphae, 3.7–12.5 μm diam., some slightly inflated, with light brown content, septate, interwoven. CLAMP CONNECTIONS absent in all parts examined.

Volvariella spp. nov. (Brazil) ... 395

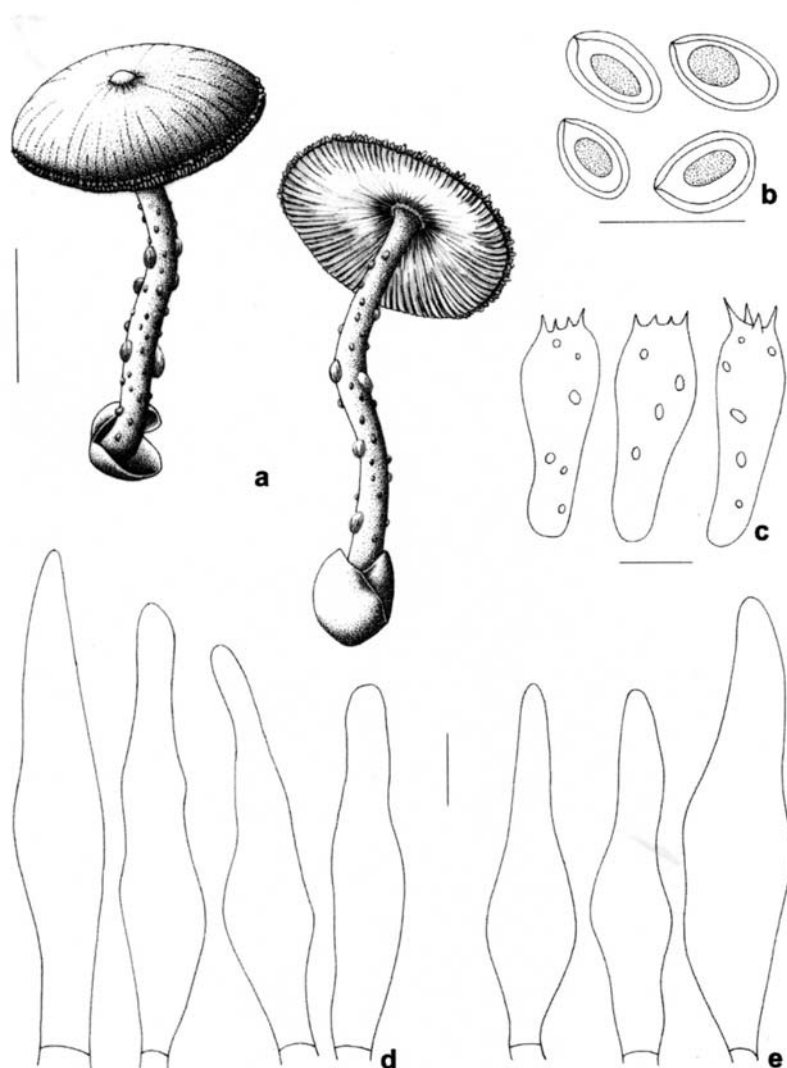
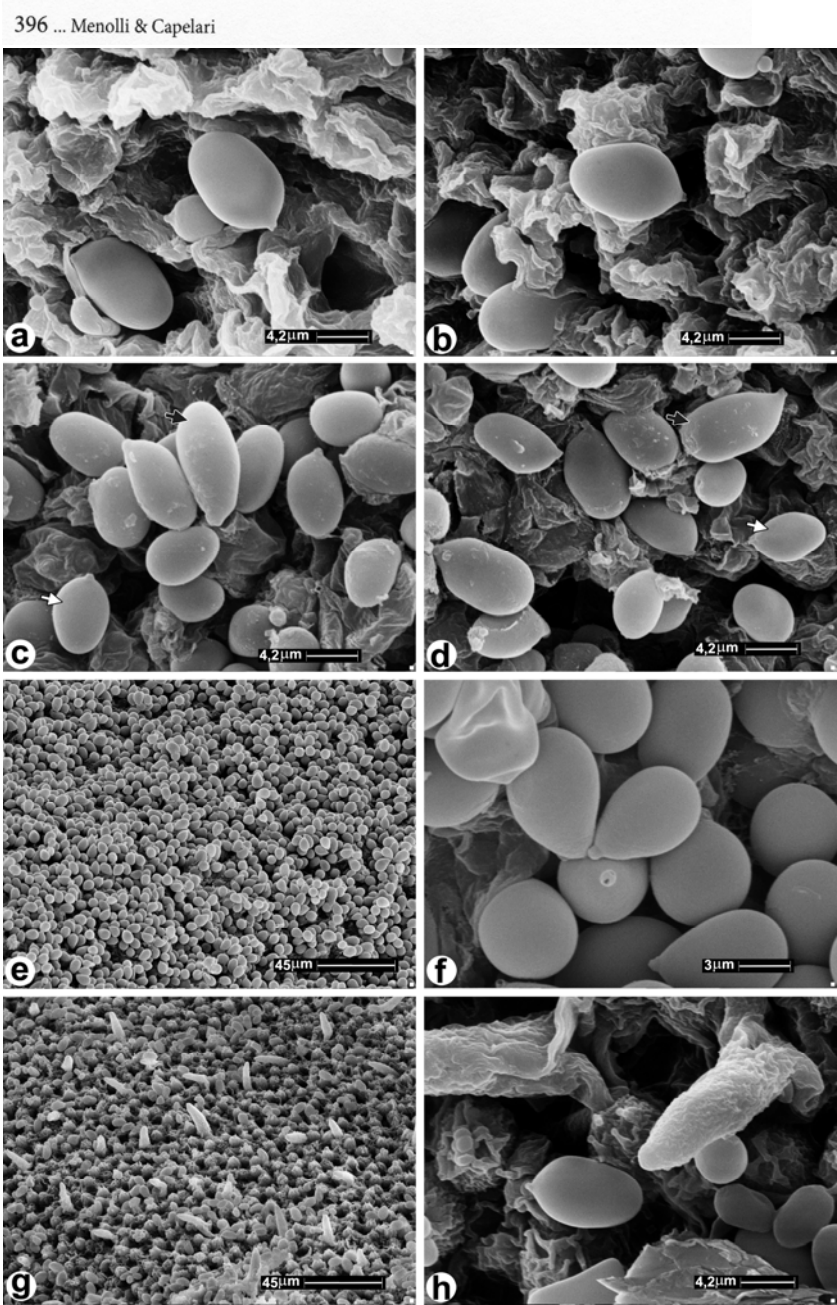


FIGURE 4: *Volvariella perciliata*.
 a. Basidioma with many humidity drops over stipe surface (scale bar = 1 cm).
 b. Basidiospores. c. Basidia. d. Pleurocystidia. e. Cheilocystidia. Scale bars = 10 µm.



Volvariella spp. nov. (Brazil) ... 397

HABITAT AND SUBSTRATE — Solitary on wood or litter.

SPECIMENS EXAMINED — BRAZIL. SÃO PAULO STATE: São Paulo, PARQUE ESTADUAL DA CANTAREIRA, NÚCLEO ENGORDADOR — 20.III.2007, *Menolli Jr. et al.* 115 (SP); 31.I.2008, *F. Karstedt et al.* 1051 (SP).

COMMENTS — *Volvariella perciliata* was originally described from French Guyana (Courtecuisse 1991) and is characterized by a small basidioma, a convex grayish-brown pileus with a mammillate umbo and a ciliate margin throughout its extension. The Brazilian specimen has basidiospores that are slightly longer than those described in the protologue ($7-8 \times 4-4.5 \mu\text{m}$) and pleurocystidia slightly shorter than the type ($45-75 \times 7-12 \mu\text{m}$), complying in all other characteristics. Since its original description, this species has been recorded for Paraná State (Meijer 2006). This collection represents the first record for São Paulo State and the second record for Brazil.

Acknowledgments

The authors thank Dr. Clark L. Ovrebo, University of Central Oklahoma, and Dr. Régis Courtecuisse, University of Lille, for critical review of the manuscript; Dr. Shaun R. Pennycook, Landcare Research, for nomenclature review; Dr. Jefferson Prado, Instituto de Botânica, who kindly revised the Latin diagnoses; Fernanda Karstedt, Instituto de Botânica, for collecting some specimens and assistance with SEM; Anderson Luis dos Santos, Instituto de Botânica, for assistance with formatting the plates; Klei R. Sousa for assistance with ink illustrations; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the support and grant to the first author; and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant 04/04319-2) for financial support.

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FIGURE 5: SEM images. a–b. Basidiospores of *Volvariella bombycina*. c–d. Basidiospores of *V. heterospora* (black arrow: large basidiospores; white arrow: small basidiospores). e–f. *V. nullicystidiata*. e. Surface of lamella showing the absence of cystidia. f. Basidiospores. g–h. *V. perciliata*. g. Surface of lamella with numerous pleurocystidia. h. Basidiospores and pleurocystidia.

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DISCUSSÃO GERAL

Este trabalho possibilitou ampliar o conhecimento das espécies de *Amanitaceae* e de *Pluteaceae* nos três remanescentes de Mata Atlântica estudados, contribuindo também com o conhecimento de sua ocorrência e distribuição em áreas de Mata Atlântica no estado de São Paulo e no Brasil.

Foram analisadas 47 exsicatas das famílias *Amanitaceae* e *Pluteaceae* coletadas no período compreendido entre janeiro/2007 a dezembro/2007, ou posteriormente a essa data em coletas esporádicas, ou ainda materiais que já estavam depositados no herbário SP (Instituto de Botânica). Além disso, quando necessário, materiais adicionais de outras localidades ou o holótipo de espécies de interesse foram analisados. *Pluteus cervinus* var. *griseoviridis*, com tipo descrito para o PEC, não foi localizado no herbário SP.

Nas áreas em estudo, a família *Amanitaceae* foi representada por uma única espécie de *Amanita*, enquanto que para *Pluteaceae* foram registradas quatro espécies de *Volvariella* e 14 espécies de *Pluteus*. *Catatrampa*, *Chamaeota* e *Limacella* não foram encontrados.

A obtenção de seqüências de nLSU e/ou ITS foi possível para as espécies dos três gêneros registrados nas áreas de coleta. No entanto, um estudo mais aprofundado de filogenia molecular só foi possível de ser conduzido com as espécies de *Pluteus*, por se tratar de um grupo bem representado nas três áreas de coleta e com um número razoável de seqüências disponíveis no GenBank (banco de dados público de seqüências de DNA). Para *Amanita*, por apenas uma espécie ter sido coletada, não foi necessário fazer qualquer tipo de análise que complementasse o atual conhecimento das relações filogenéticas do gênero. E, para as espécies de *Volvariella*, por se tratar de um gênero pequeno, sem propostas de classificação infragenérica e sem grande disponibilidade de seqüências no GenBank, também não foi possível conduzir nenhum tipo de análise molecular. No entanto, nestes dois casos, *Amanita* e *Volvariella*, as seqüências obtidas foram disponibilizadas no GenBank e os respectivos números de acesso foram atrelados às publicações apresentadas neste trabalho.

Para o gênero *Amanita*, a única espécie coletada foi *A. coacta*, registrada somente para o PEFI. A partir dos materiais recentemente coletados foi possível complementar a descrição original da espécie, anteriormente conhecida somente da localidade tipo (Amazonas), além de disponibilizar seqüências de nLSU.

As espécies de *Volvariella*, *V. bombycina*, *V. heterospora*, *V. nullicystidiata* e *V. perciliata*, foram restritas ao PEC. Destas, *V. heterospora* e *V. nullicystidiata* representam espécies novas para a ciência, e *V. perciliata* é nova citação para o estado São Paulo e a segunda citação para o Brasil. *Volvariella bombycina* é uma nova citação para o PEC.

Seqüências de nLSU e ITS foram disponibilizadas para *V. bombycina*, *V. nullicystidiata* e *V. perciliata*.

Dentre as 14 espécies de *Pluteus* levantadas, foi possível obter seqüências de genes para filogenia molecular de 10 espécies para as áreas de estudo. Para as outras quatro espécies não foi possível obter quantidade suficiente de material amplificado para o seqüenciamento, ou não havia material liofilizado disponível, por se tratarem de basidiomas geralmente solitários e pequenos. Mesmo assim, a partir de estudos morfológicos destas quatro espécies, foi registrada a ocorrência de duas novas espécies, *P. bulbomarginatus* e *P. concavus*, além de uma nova citação para o Brasil (*P. longistriatus*) e uma nova citação para o estado São Paulo representada pela segunda coleta de *P. umbrinoalbidus* desde a descrição original do material coletado no Rio Grande do Sul.

Dentre as demais espécies de *Pluteus* identificadas nas áreas de estudo, duas espécies novas, *P. aureovenatus* e *P. capillicomptus*, e uma variedade nova, *P. dominicanus* var. *hyalinus* foram descritas. Outras três espécies, *P. fuligineovenosus*, *P. jamaicensis* e *P. riberaltensis* var. *conquistensis*, constituem novas citações para o Brasil. Além disso, *P. fluminensis* é uma nova citação para o PEFI, *P. harrisii* citação nova para o PEC e para a RBP, e *P. xylophilus* é ocorrência nova para o PEC.

Os estudos moleculares com as espécies de *Pluteus* permitiram uma maior compreensão da classificação infragenérica proposta para o gênero e contribuíram para inserção de espécies brasileiras na filogenia global dos basidiomicetos. Estes estudos também deram suporte para diferenciar espécies morfológicamente relacionadas, difícil de serem distinguidas apenas com estudos morfológicos. Neste sentido, foi possível estabelecer um status novo em nível específico para *P. sublaevigatus*.

A distribuição das espécies de *Pluteus* nas três áreas de coleta revelou uma maior preponderância de espécies no PEC, totalizando onze espécies, sendo nove de ocorrência exclusiva em comparação às demais áreas amostradas: *P. bulbomarginatus*, *P. capillicomptus*, *P. concavus*, *P. dominicanus* var. *hyalinus*, *P. fluminensis*, *P. fuligineovenosus*, *P. harrisii*, *P. jamaicensis*, *P. sublaevigatus*, *P. umbrinoalbidus* e *P. xylophilus*.

Para a RBP, *P. harrisii* foi a única espécie de *Pluteaceae* e *Amanitaceae* registrada, sendo esta espécie também de ocorrência comum ao PEC. Para o PEFI foram registradas quatro espécies de *Pluteus*, sendo três delas exclusivas para a área (*P. aureovenatus*, *P. longistriatus* e *P. riberaltensis* var. *conquistensis*) e uma (*P. xylophilus*) de ocorrência comum ao PEC.

A grande diferença de distribuição das espécies observada nas três áreas amostradas pode estar diretamente relacionada ao estado de conservação dos remanescentes florestais.

Assim, a proximidade da RBP com o pólo industrial de Cubatão e o alto grau de poluição atmosférica observado no passado, pode ter influenciado significativamente na diminuição da diversidade fúngica na região. Também, a intensa influência antrópica e a grande proximidade do PEFI com áreas intensamente urbanizadas e municípios de grande desenvolvimento industrial, podem ser responsáveis pelo pequeno número de espécies registradas para a área, quando comparadas ao PEC, que, apesar do acelerado processo de urbanização observado em seu entorno, ainda apresenta uma cobertura vegetal em bom estado de conservação.

Este trabalho não esgota o registro das espécies de *Pluteaceae* e *Amanitaceae* para as áreas estudadas, mas contribui significativamente para ampliar o conhecimento dessas famílias no estado de São Paulo e no Brasil, e, de forma pioneira, com a inclusão destas espécies em trabalhos utilizando biologia molecular.

APÊNDICE



Pluteus aureovenatus Menolli & Capelari



Pluteus capillicomptus Menolli & Capelari



Pluteus dominicanus var. *hyalinus* Menolli & Capelari



Pluteus fluminensis Singer



Pluteus fuligineovenosus Horak



Pluteus harrisii Murrill



Pluteus jamaicensis Murrill



Pluteus riberaltensis var. *conquistensis* Singer



Pluteus sublaevigatus (Singer) Menolli & Capelari



Pluteus xylophilus (Speg.) Singer



Volvariella bombycina (Schaeff.) Singer



Volvariella heterospora Menolli & Capelari



Volvariella nullicystidiata Menolli & Capelari



Volvariella perciliata Courtec.

CRÉDITO DAS IMAGENS

CAPA – Arte final: Luiz Antonio Silva Ramos.

Aspecto geral da vegetação da RBP – foto Fernanda Karstedt.

Pluteus concavus – foto Fernanda Karstedt.

Volvariella nullicystidiata – foto Fernanda Karstedt.

Pluteus fluminensis – fotos Fernanda Karstedt.

Volvariella perciliata – foto Fernanda Karstedt.

Pluteus aureovenatus – foto Fernanda Karstedt.

Pluteus capillicomptus – foto Marina Capelari.

Pluteus sp. – foto Fernanda Karstedt.

Volvariella bombycina – foto Fernanda Karstedt.

Amanita coacta – fotos Marina Capelari e Nelson Menolli Junior.

Pluteus sublaevigatus – fotos Marina Capelari.

PÁG. 18

Figura 4b: Aspecto geral da vegetação do PEC – foto Nelson Menolli Junior.

PÁG. 19

Figura 5b: Aspecto geral da vegetação do PEC – foto Nelson Menolli Junior.

PÁG. 20

Figura 6b: Aspecto geral da vegetação da RBP – foto Fernanda Karstedt.

PÁG. 33

Figura 1: *Amanita coacta*. (a; e–h) fotos Fernanda Karstedt. (b–d; i–l) fotos Marina Capelari.

PÁG. 46

Figura 1a–g: (a–b) *Pluteus bulbomarginatus* – fotos Fernanda Karstedt. (c–d) *Pluteus concavus* – fotos Fernanda Karstedt. (e) *Pluteus longistriatus* – fotos Fernanda Karstedt. (f–g) *Pluteus umbrinoalbidus* – fotos Fernanda Karstedt.

PÁG. 117

Pluteus aureovenatus – fotos Fernanda Karstedt.

Pluteus capillicomptus – fotos Fernanda Karstedt.

PÁG. 118

Pluteus dominicanus var. *hyalinus* – fotos Fernanda Karstedt.

Pluteus fluminensis – fotos Fernanda Karstedt.

PÁG. 119

Pluteus fuligineovenosus – fotos Fernanda Karstedt.

Pluteus harrisii – fotos Fernanda Karstedt.

PÁG. 120

Pluteus jamaicensis – fotos Fernanda Karstedt.

Pluteus riberalensis var. *conquistensis* – fotos Fernanda Karstedt.

PÁG. 121

Pluteus sublaevigatus – fotos Marina Capelari.

Pluteus xylophilus – fotos Nelson Menolli Junior.

PÁG. 122

Volvariella bombycina – fotos Fernanda Karstedt.

Volvariella heterospora – fotos Fernanda Karstedt.

PÁG. 123

Volvariella nullicystidiata – fotos Fernanda Karstedt.

Volvariella perciliata – fotos Fernanda Karstedt.