#### TAXONOMIA E FILOGENIA PRELIMINAR DE AMAURODERMA (GANODERMATACEAE, POLYPORALES)

Dissertação submetida ao Programa de Pós Graduação em Biologia de Fungos, Algas e Plantas da Universidade Federal de Santa Catarina para a obtenção do Grau de mestre em Biologia de Fungos, Algas e Plantas. Orientador: Elisandro Prof. Dr. Ricardo Drechsler dos Santos. Coorientador: Prof. Drª. Adriana de Mello Gugliotta.

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## "Taxonomia e filogenia preliminar de Amauroderma (Ganodermataceae, Polyporales)"

por

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Florianópolis, 29 de maio de 2014.

Este trabalho é dedicado aos meus queridos pais, Adão e Nilza.

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Muito obrigado.

Mudam-se as técnicas e as tendências, mas a taxonomia seguirá sua trilha de séculos: interpretar as entidades que habitam o planeta e legislar sobre seus nomes.

João Salvador Furtado, 1968

#### RESUMO

Amauroderma é um gênero de macrofungos poliporoides de Ganodermataceae caracterizado por apresentar basidiomas sésseis a estipitados, com coloração em diferentes tons de marrom, sistema hifal dimítico, basidiósporos globosos, elipsoides a oblongos, hialinos a amarelados e com dupla parede, onde o endósporo é ornamentado (uma espécie apresenta endósporo liso). O gênero está distribuído em regiões tropicais e subtropicais, geralmente associados a raízes de árvores vivas ou mortas, mas também ocorrendo em troncos mortos. Apesar de bem delimitado morfologicamente, nenhuma análise filogenética do gênero foi realizada até o momento e suas relações em Ganodermataceae são assumidas por apenas três espécies. Este trabalho apresenta aspectos taxonômicos e filogenéticos sobre Amauroderma, a partir de análises morfológicas (macro, micro e ultraestrurais) bem como filogenéticas moleculares. Ao todo, foram revisados 144 espécimes (coletas e coleções de herbário), representando 17 espécies de Amauroderma e cinco de gêneros relacionados. Dentre estes, duas novas espécies são propostas, Amauroderma calcitum Ad int. com morfologia intermediária a A. partitum e A. calcigenum, e A. opacaespitosa Ad int., caracterizada por apresentar hábito cespitoso, basidiomas opacos com contexto marrom, sem linhas e esporos amplamente elipsoides. Também é proposta a sinonimização de A. omphalodes em A. camerarium, bem como são apresentados novos registros para o domínio fitogeográfico do Cerrado e para a Argentina. Por fim, é apresentada uma filogenia (ITS, LSU e ITS+LSU) com a proposição de um gênero novo (Foraminisporus Ad int.) e uma nova combinação (F. sprucei Ad int.), caracterizados por apresentar colunas endospóricas ocas, sendo esta uma sinapomorfia dos táxons. Ainda, Amauroderma é considerado um táxon polifilético. A partir dos resultados apresentados aqui, nota-se a eminente possibilidade de novidades científicas, tanto a nível específico quanto a nível genérico, bem como a necessidade de estudos que envolvam análises morfológicas (macro, micro e ultraestrutural) e moleculares com múltiplos marcadores.

Palavras-chave: Agaricomycetes. Políporos. Sistemática Filogenética.

### ABSTRACT

Amauroderma is a polypore macrofungi genus oin theGanodermataceae characterized by sessile to stipitate basidiomata, dimitic hyphal system, and globose, ellipsoid to oblong, hvaline to yellowish, double-walled basidiospores with ornamented endospore (one species presents smooth endospore). Amauroderma occurs in tropical and sub-tropical regions, usually associated with roots of living or dead trees, also occurring in dead trunks. Despite its well morphological circumscription Amauroderma is phylogenetically understudied and its relationship in Ganodermataceae is based on few species. A taxonomic and phylogenetic approach of Amauroderma is presented, based on morphological (macro, micro and ultrastructure) and molecular analysis. Around 144 specimens (collections and herbarium material). representing 17 species of Amauroderma and five of related genera were studied. Two new species are proposed, Amauroderma calcitum Ad int. with intermediate morphology of A. partitum and A. calcigenum, and A. opacaespitosa Ad int., characterized by caespitose habbit, dull basidiomata with brown context without bands and broadly ellipsoid basidiospores. Furthermore, the synonym of A. omphalodes in A. camerarium is also proposed, as well as new records for Brazilian Cerrado and for Argentina. Finally, in a preliminary phylogenetic point of view Amauroderma is considered polyphyletic. Considering the results presented here is possible to observed that there are many scientific novelties among the Ganodermataceae (specific and generic level) and some of them are possible to see only using a detailed morphology combined with molecular studies.

Keywords: Agaricomycetes. Polypores. Phylogenetic systematics.

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## 1. APRESENTAÇÃO

Neste trabalho é apresentada uma filogenia preliminar de *Amauroderma* Murrill, bem como a proposição de novos táxons e ampliação da distribuição geográfica de algumas espécies. Está subdividido em Introdução (Fundamentação Teórica), Objetivos, Materiais & Métodos, Resultados & Discussão e Conclusão. Os Resultados & Discussão estão subdivididos em capítulos, onde:

Capítulo I – é apresentada a proposição de uma nova espécie de *Amauroderma*, bem como a sinonimização de *A. omphalodes* (Berk.) Torrend em *A. camerarium* (Berk.) J.S. Furtado e novos registros do gênero para o domínio Cerrado, em formato de artigo a ser submetido à revista *Phytotaxa*;

Capítulo II – é apresentada a proposição de uma nova espécie, bem como notas em espécies do gênero que ocorrem na Argentina, em formato de artigo a ser submetido à revista Mycoscience;

Capítulo III – é apresentada uma filogenia preliminar de *Amauroderma*, com a proposição de um novo gênero e uma nova combinação, em formato de artigo a ser submetido à revista *Mycologia*.

Todas as referências bibliográficas citadas na Introdução e Material & Métodos são apresentadas após a conclusão e as referências dos demais capítulos são apresentadas no final de cada um deles, bem como uma tabela com todos os espécimes examinados é apresentada no final da dissertação.

## 2. INTRODUÇÃO

## 2.1 REINO FUNGI: BREVE CARACTERIZAÇÃO

O Reino Fungi é um grupo megadiverso, com estimativas que chegam a 5,1 milhões de espécies (Hawksworth 1991; Blackwell 2011). O reino compreende organismos eucariontes, unicelulares ou multicelulares, heterótrofos por absorção, dotados de parede celular, leveduriformes e filamentosos, podendo ainda apresentar estruturas reprodutivas macroscópicas. Os fungos estão presentes nos mais variados habitats da superfície terrestre, desempenhando papel fundamental para manutenção da vida na terra, tanto ao degradar a matéria morta (sapróbios), atuando na ciclagem de nutrientes, quanto como simbiontes, associados aos mais variados organismos, desde artrópodes a seres humanos, plantas e até mesmo outros fungos. (Mueller et al. 2004; Webster & Weber 2007; Moore et al. 2011). Os fungos são organismos de grande importância econômica, sendo amplamente utilizados pelo homem nos setores alimentício, medicinal, industrial e agrícola, participando desde processos de fermentação (pães, queijos e bebidas alcóolicas), produção de antibióticos, biorremediação, fungicidas e biopesticidas, entre muitos outros, além de causarem danos a vários cultivos agrícolas (Esposito & Azevedo 2010; Moore et al., 2011).

## 2.2 GANODERMATACEAE DONK: CARACTERIZAÇÃO E BREVE HISTÓRICO

Ganodermataceae Donk (Polyporales Gäum, Agaricomycetes Doweld) é caracterizada principalmente por apresentar basidiomas pileados, sésseis a estipitados, poroides, sistema hifal di-trimítico com presença de hifas esqueletais arboriformes e/ou esqueleto ligadoras e pela presença de basidiósporos com parede dupla, onde a interna apresenta ornamentação, sendo esta, uma característica exclusiva do táxon (Furtado 1962; Ryvarden 2004). A família foi proposta em 1948 para compreender os gêneros *Amauroderma* Murril e *Ganoderma* Karst, previamente pertencentes à subfamília Ganodermatoideae Donk de Polyporaceae Corda (Furtado 1981; Moncalvo & Ryvarden 1997). Ganodermataceae apresenta distribuição cosmopolita, com variação aproximada de 117 a 220 espécies descritas, de acordo com Kirk et al.

(2010) e Moncalvo & Ryvarden (1997), respectivamente. As espécies são causadoras de podridão branca na madeira ou estão associadas a raízes de árvores vivas ou mortas (Furtado 1981; Ryvarden 2004).

Historicamente, a taxonomia da família é baseada quase que exclusivamente em atributos morfológicos, como presença de laca na superfície do píleo, disposição e composição do sistema hifal (contexto, trama dos tubos e cutícula dos basidiomas) e entre outras características dos esporos a forma e o padrão de ornamentação (Torrend 1920; Furtado 1962; Furtado 1965, 1981; Steyart 1972; Ryvarden & Johansen 1980; Corner, 1983; Gottlieb & Wright 1999 a,b; Ryvarden 2004; Torres-Torres & Guzmán-Dávalos 2012). Com base nesses caracteres, muitas discussões foram levantadas sobre o status genérico e infragenérico em Ganodermataceae.

Além de Amauroderma e Ganoderma, os gêneros Haddowia Steyaert, Humphreya Steyaert e Tomophagus Murrill são aceitos por autores modernos (Moncalvo 1996; Moncalvo & Ryvarden 1997; Ryvarden 2004; Kirk et al. 2008; Le et al. 2011). Ganoderma é caracterizado principalmente por apresentar esporos elipsoides à ovoides de ápice truncado, com ornamentação colunar. Tomophagus apresenta esporos similares, diferindo por apresentar contexto macio. Humphreya apresenta esporos com mesmo formato, diferindo principalmente por apresentar ornamentação em forma de crestas Amauroderma predominantemente longitudinais. e Haddowia apresentam esporos sem o ápice truncado, diferindo entre si pelo padrão de ornamentação, ou seja, ornamentação colunar a semi-reticulada e em forma de crestas longitudinais, respectivamente (Furtado 1981; Stevaert 1972; Ryvarden 2004).

Além destes, outros gêneros com esporos ganodermatoides foram propostos, porém amplamente reconhecidos como sinônimos de *Ganoderma* ou *Amauroderma* (Ryvarden 1991; Moncalvo & Ryvarden 1997). *Elfvingia* P. Karst. foi proposto para contemplar as espécies com esporos similares aos de *Ganoderma*, mas com superfície do píleo opaca. Isto restringiria as espécies com superfície do píleo lacada a *Ganoderma*, característica observada na espécie tipo do gênero, *G. lucidum* (Curtis) P. Karst. Por fim, *Magoderma* Steyaert apresenta esporos sem o ápice truncado, diferindo de *Amauroderma* por apresentar hifas subcuticulares e cuticulares de conformação anticlinal.

Ainda, alguns subgêneros e sessões foram propostos. Steyaert propôs os subgêneros *Anamixoderma* Steyaert e *Plecoderma* Steyaert, baseados principalmente em diferenças microestruturais da superfície do píleo (ambos dentro de *Ganoderma*) e *Elfvingia* (Karst.) Imazeki foi redefinida como subgênero por Imazeki. As sessões *Amauroderma* Pat., hoje amplamente aceita como gênero *sensu* Murrill, *Phaeonema* Zhao, Xu & Zhang, caracterizada por apresentar superfície do píleo lacada e contexto marrom homogêneo, e *Characoderma* Steyaert, caracterizada também a partir das microestruturas da superfíce do píleo (todas em *Ganoderma*).

Poucos trabalhos tratam das relações filogenéticas da família e ainda são pouco inclusivos, visto que utilizam vários táxons de *Ganoderma* e poucos representantes ou nenhum dos demais gêneros. As análises filogenéticas realizadas até o momento têm apresentado resultados divergentes, onde a família aparece como monofilética, parafilética e polifilética (Moncalvo et al.1995; Moncalvo 1996, Hong & Jung 2004; Justo & Hibbett 2011; Binder et al. 2013).

### 2.3 HISTÓRICO DO CONCEITO DE AMAURODERMA Murrill

O nome *Amauroderma* foi utilizado pela primeira vez por Patouillard (1889), quando seccionou o gênero *Ganoderma* em dois. A seção *Ganoderma* compreendia espécies com basidiósporos ovóides, truncados e superfície do píleo lacada e a seção *Amauroderma* espécies com basidiósporos globosos ou subglobosos sem base truncada e píleo não lacado. Murril em 1905 elevou *Amauroderma* a gênero, com *A. regulicolor* (Berk. ex Cooke) Murrill como espécie tipo, com a seguinte circunscrição: himenóforo grande, sobre madeira, estipe geralmente muito alongado, superfície lisa, encrustrada, não lacada, macio, tubos cilíndricos, poros inicialmente de cores claras e esporos marrons, ovoides ou globosos.

Depois da proposição do gênero, varias espécies foram estudadas por Lloyd (Mycological writings e Mycological Letters 1912-1920), no entanto como *Polyporus* seção *Amaurodermus*. Torrend (1920) apresentou uma das maiores contribuições para o conhecimento do gênero *Amauroderma* para a América do Sul, ao descrever novos táxons e revisar as notas de Lloyd e elevar a seção proposta por Pattouillard (1889) a gênero. No entanto, essa proposição não é válida, pois Pattouillard não citou uma espécie tipo para a seção, sendo válida a proposição de Murrill. Tanto Lloyd quanto Torrend apresentaram espécies com esporos ornamentados e lisos para o gênero. Furtado (1962), a partir de análises morfológicas (micro e ultraestrutural), observou que a ornamentação ocorre no endósporo da parede dupla dos esporos de *Amauroderma*.

Steyaert (1972) além de apresentar três novos gêneros em *Ganodermataceae*, observou que a circunscrição de *Amauroderma* era muito ampla e certamente deveria ser artificial. Segundo o autor, o gênero apresenta muita variação tanto na morfologia do esporo quanto na composição hifal do contexto e superfície do píleo. Sendo assim, separou as espécies do gênero em três morfogrupos, baseado principalmente na composição hifal da superfície do píleo, onde as espécies sem diferenciações seriam as representantes do núcleo de *Amauroderma*. Dentre os gêneros, *Magoderna* foi proposto para compreender duas espécies (*Amauroderma infundibiliforme* Wakef e *Fomes subresinosus* Murrill) com hifas cuticulares com disposição anticlinal. No entanto, atualmente o gênero é considerado um sinônimo de *Amauroderma* (Furtado 1981; Corner 1983).

Poucos trabalhos foram realizados no continente africano, dentre eles destacam-se o trabalho de Otieno (1968), onde uma descrição simples do gênero é apresentada, bem como algumas algumas espécies com esporos lisos. Atualmente, sabe-se que todas as espécies que Otieno apresentou com esporos lisos, apresentam na verdade, endósporo ornamentado. Ryvarden & Johansen (1980) também ao estudar alguns espécimes da África, apresentam um conceito muito similar com o de Furtado (1965, 1968 discutido a seguir), diferindo apenas ao excluirem *A. longipes* (Lev.) Torrend do gênero. Atualmente esta espécie está posicionada em *Haddowia, H. longipes* (Lev.) Steyaert.

Uma revisão do gênero foi apresentada por Furtado [1981, resultado de uma compilação de novos dados e pesquisas anteriores (1965,1968)], com 140 táxons, sendo 30 excluídos do gênero por não apresentarem os basidiósporos característicos. Dos 110 restantes muitos foram sinonimizados e 27 espécies foram apresentadas. Segundo o autor, que considerou o gênero em Polyporaceae, todos os representantes do grupo são estipitados, de consistência papirácea a amadeirada, sistema hifal trimítico e esporos globosos a elipsoides, raramente oblongos, nunca truncados, com endósporo ornamentado. O autor considera apenas dois gêneros ganodermatoides (*Amauroderma* e *Ganoderma*) ambos com uma circunscrição mais ampla do que a proposta por Steyaert (1972). A principal diferença do conceito de *Amauroderma* de Furtado para o mais aceito atualmente (Ryvarden 2004) é a presença de *A. longipes*, que apresenta esporos globosos a

subglobosos com ornamentação em forma de crestas longitudinais. Ryvarden (2004) reconhece *Haddowia longipes*.

Corner (1983) apontou que os gêneros de *Ganodermataceae* apresentam espécies com várias características em comum, e que uma diferenciação genérica baseada principalmente na morfologia dos esporos e composição hifal da superfície do píleo pode não ser natural. Por isso, Corner sugere que o conceito de Pattouillard (1889) esteja certo, onde *Amauroderma* deveria ser uma seção de *Ganoderma*. Heim (1962) concorda com esta sugestão. No entanto, por conveniência, o autor apresenta os quatro gêneros, *Amauroderma, Ganoderma, Haddowia* e *Humphreya*. Corner enfatiza ainda que o estudo do sistema hifal deve ser primordial para a classificação dos políporos, bem como a observação de materiais frescos e o estudo de esporadas.

Zhao (1989) apresenta 20 espécies do gênero para a China, incluindo várias espécies anteriormente propostas por ele, considerando também espécies com esporos lisos e algumas que até então eram consideradas neotropicais. Dai (2011), por sua vez, apresenta uma listagem com as espécies de políporos da China, citando apenas seis espécies de *Amauroderma*, sem discutir a 'exclusão' das outras 14. Esta diferença no número de espécies citadas por Dai e Zhao aponta a necessidade da revisão das coleções de *Amauroderma* da China, para real conhecimento da diversidade do gênero.

Ryvarden (2004) estudou 21 espécies neotropicais, apresentando táxons novos e novas combinações. O conceito do autor apresenta algumas diferenças do estabelecido por Furtado (1981), principalmente porque considera uma espécie com basidiomas sésseis em *Amauroderma (A. andinum* Ryvarden) e outra com esporos lisos (*A. coltricioides* Aime, Henkel & Ryvarden), mas exclui *A. longipes*, assim como Ryvarden & Johansen (1980), Corner (1983) e Zhao (1989).

Nenhuma análise filogenética do gênero foi realizada até o momento e suas relações filogenéticas em Ganodermataceae são assumidas com base em apenas três espécies. De modo geral, os resultados tem sido divergentes. Moncalvo et al. (1995) e Moncalvo (1996), a partir das regiões ITS e LSU, utilizaram o táxon *Amauroderma rude* (Berk.) Torrend, que ficou posicionado como um clado irmão de *Ganoderma*. Recentemente, Glen et al. (2009) apresentam uma filogenia (rDNA ITS) onde foram utilizadas as espécies *Amauroderma rude* e *Amauroderma subrugosum* (Bres. & Pat.) Torrend, porém ao contrário dos trabalhos anteriores, as espécies de *Amauroderma* agruparam-se dentro de um dos clados de *Ganoderma*.

No entanto, os autores explicam que isso pode ter ocorrido em virtude de a região selecionada para o estudo ser menos conservada. Neste caso, os resultados não são confiáveis para uma relação filogenética entre gêneros. Justo & Hibbett (2012), em seu trabalho sobre fungos trametóides, propuseram duas filogenias baseadas em dois (nLSU e ITS) e cinco genes (nLSU, ITS, RPB1, RPB2 e TEF1), onde *A. rugosum* (Blume & T. Nees) Torrend apareceu mais relacionado a *Coriolopsis* cf. *byrsina* (Mont.) Ryvarden do que ao clado composto por espécies de *Ganoderma*. Um resultado semelhante foi obtido por Binder et al. (2013) onde *A. rugosum* mostrou-se mais relacionado a *Perenniporia* Murrill s.l. do que com *Ganoderma*. Portanto, a relação filogenética entre os gêneros da família, e da própria família em relação a outros táxons, ainda não é clara e deveria ser melhor explorada.

A circunscrição de *Amauroderma* mais recente (Ryvarden 2004) compreende espécies com basidiomas sésseis a estipitados, com coloração em diferentes tons de marrom, sistema hifal dimítico, com hifas generativas fibuladas e esqueletais arboriformes, variavelmente dextrinoides, basidiósporos globosos, elipsoides a oblongos, com dupla parede onde o endósporo é ornamentado (uma espécie apresenta endósporo liso), hialinos a amarelados e variavelmente dextrinoides. O gênero está distribuído em regiões tropicais e subtropicais, geralmente associados a raízes de árvores vivas ou mortas, mas também ocorrendo em troncos mortos (Torrend 1920; Ryvarden & Johansen 1980; Furtado 1981; Moncalvo & Ryvarden 1997; Ryvarden 2004).

### **3. OBJETIVOS**

## 3.1 OBJETIVO GERAL

O objetivo geral deste trabalho é apresentar aspectos taxonômicos e filogenéticos do gênero *Amauroderma* com ênfase nas espécies que ocorrem no Brasil.

## 3.2 OBJETIVOS ESPECÍFICOS

- Revisar macro e micromorfologicamente as espécies, a partir de espécimes representativos, incluindo materiais tipo (quando possível) e de referência para cada táxon;
- Extrair, amplificar e sequenciar as regiões ITS e 28S do rDNA nuclear de materiais coletados (Brasil e Argentina) e de referência;
- Interpretar filogeneticamente as espécies a partir das análises morfológicas, ultra-estruturais e moleculares.

### 4. MATERIAIS E MÉTODOS

### 4.1 COLEÇÕES EXAMINADAS E PROCESSAMENTO DO MATERIAL

Foram estudados espécimes de *Amauroderma* coletados em ecossistemas do Cerrado (MT) e da Mata Atlântica (SC, ES, BA, PB), bem como nas Yungas Argentinas, tanto realizadas para esse trabalho como por colaboradores no âmbito de outras pesquisas. Foram também analisados materiais tipo e de referência depositados nos herbários BPI, CANB, CORD, FH, FLOR, INPA, K, NY, JPB, UEFS e URM. Os basidiomas coletados foram levados para o laboratório e em seguida mantidos por 12 à 24h em estufa para desidratação (30° a 35°C), foram também armazenados fragmentos dos basidiomas em tubos tipo *eppendorfs* com sílica, a fim de preservar o DNA para estudos moleculares. Todos os espécimes estudados estão listados na tabela I e todos os espécimes coletados estão de acordo com a base de dados *Index Herbariorum* (Thiers, atualizado continuamente).

## 4.2 ANÁLISES MORFOLÓGICAS

descrição macroscópica foi observado Para а hábito 0 (séssil/estipitado), sazonalidade. dimensões do basidioma (contexto/tubos), bem como caracteres do himenóforo (poros: tipo/formato, tamanho e nº por milímetro linear; dissipimentos: tamanho. características), do substrato (hospedeiro: possível identificação e condição viva ou morta) e a determinação das cores está de acordo com o catálogo de Munsell (1975).

Para observação dos caracteres microscópicos foram realizados cortes dos basidiomas (superfície do píleo, contexto e dos tubos) à mão livre para estudo dos tipos de hifas (sistema hifal), presença ou ausência de elementos estéreis, basídios e basidiósporos. A descrição dos basidiósporos segue o modelo de Furtado (1962). Os cortes foram montados em lâminas e lamínulas: KOH 2-3% (hidratante para observação da coloração das hifas e basidiósporos), Floxina 1% (corante para observar as características das hifas generativas, himênio e basidiósporos) e reagente de Melzer (para observar a presença ou ausência das reações dextrinoide e amiloide nas hifas e basidiósporos), bem como fragmentos do basidioma foram incubados por 24-48 horas

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em NaOH 3%, com posterior dissecação em estéreo microscópio (Teixeira 1995; Decock 2010). A observação e mensuração (n=40) das estruturas foram realizadas em microscópio óptico com ocular micrométrica, 5% das medidas de cada extremo são apresentadas entre parênteses, as abreviações seguem Amalfi et al. (2010).

## 4.3 ANÁLISE ULTRAESTRUTURAL

Para observação do padrão de ornamentação dos esporos foi realizada a análise ultraestrutural em Microscópio Eletrônico de Varredura (MEV) JEOL JSM-6390LV do Laboratório Central de Microscopia Eletrônica da Universidade Federal de Santa Catarina e Zeiss LEO 1450VP do Laboratorio de Microscopia Electronica y Microanalisis (LABMEM) da Universidad Nacional de San Luis, Argentina. Para uma análise comparativa, foram observados esporos com e sem o exosporo. Para a remoção do exosporo, fragmentos dos tubos foram colocados em vidro-relógio com cristais de ácido crômico (H2CrO4), em seguida cobertos com algumas gotas de água (suficientes para dissolver os cristais) e reservadas por 20 minutos. Depois disso, a solução e os fragmentos de basidioma foram filtrados a vácuo (filtro de 0,45 µm) agregando-se ainda água destilada para remover o ácido. Por fim, depois de seco, a superfície dos filtros foi raspada para um suporte metálico (stub) com fita adesiva de grafite com uma gota de álcool 70%, e recoberto com metalização direta de ouro a 10 nm. Para observação dos esporos com exosporo a análise foi realizada através de um pequeno corte do himenóforo do basidioma, fixado em suporte metálico (stub) com fita adesiva de grafite, e recoberto com metalização direta de ouro a 10 nm

## 4.4 ANÁLISES MOLECULARES

Para as análises moleculares foram realizadas a extração e amplificação do DNA dos materiais a partir do Protocolo de Doyle & Doyle (1987) modificado por Góes-Neto et al. (2005). Para amplificação das regiões 28S e ITS (Internal Trancribed Spacer) do rDNA, foram utilizados os pares de primers LR0R/LR7 (http://www.biology.duke.edu/fungi/mycolab/primers. htm) e ITS8-F/ITS6-R (Dentinger et al. 2010), respectivamente. O sequenciamento foi realizado no Sequenciador ABI 3730 DNA Analyzer (Applied Biosystems) do Centro de Pesquisas René Rachou, Fiocruz - Belo Horizonte/MG, no âmbito do Projeto Identificação Molecular de Fungos do Brasil.

As sequências foram editadas manualmente com o software Geneious (Biomatters). Foram montadas três matrizes (ITS, LSU e ITS+LSU), contendo sequências geradas por este trabalho e algumas (http://www.ncbi.nlm.nih.gov/Genbank/). disponíveis no Genbank Todas as sequências geradas serão depositadas no mesmo banco de dados (Genbank). O alinhamento foi realizado com o software MAFFT (Katoh et al. 2009) e inspecionados manualmente no software MEGA v.6 (Tamura et al. 2013). O melhor modelo evolutivo foi selecionado com o parâmetro BIC (Bayesian Information Criterion) com o software Jmodeltest (Darriba et al. 2012; Guindon & Gascuel 2003). A análise Bayesiana foi realizada com o software BEAST v.2 (Bouckaert et al. 2014) e a de Verossimilhança com o RaxML, disponível na plataforma Cipres (maior detalhamento das análises disponível no cap. 3).

#### **5. RESULTADOS E DISCUSSÃO**

Ao todo, foram revisados 144 espécimes, representando 17 espécies de *Amauroderma* e cinco de gêneros relacionados, oriundos de coletas realizadas para este ou para trabalhos paralelos e de coleções de herbário. Vale ressaltar, que com exceção de um (1) espécime, todos os materiais coletados para esse trabalho estavam associados a raízes de plantas. A lista completa dos espécimes estudados é apresentada na Tabela I. Os resultados e a discussão são apresentados de forma separada em capítulos:

O capítulo I apresenta com base morfológica a proposição de uma espécie nova (*Amauroderma calcitum Ad int.*), com morfologia intermediária a *A. partitum* (Berk) Wakef e *A. calcigenum* (Berk.) Torrend, bem como a sinonimização de *A. omphalodes* (Berk.) Torrend em *A. camerarium* (Berk.) J.S. Furtado e novos registros do gênero para o domínio Cerrado, com ilustrações, notas taxonômicas e uma chave para as espécies que ocorrem no Brasil (Artigo a ser submetido para Phytotaxa).

O capítulo II apresenta com base morfológica a proposição de uma espécie nova (*Amauroderma opacaespitosa Ad int.*), bem como notas e uma chave para as espécies que ocorrem na Argentina (Artigo a ser submetido para Mycoscience).

O capítulo III apresenta a filogenia preliminar de *Amauroderma* com a proposição de um gênero novo (*Foraminisporus Ad int.*) e uma nova combinação (*F. sprucei Ad int.*). com base nas análises moleculares e morfológicas (macro, micro e ultraestrutural).

CAPÍTULO I - STUDIES IN AMAURODERMA (GANODERMATACEAE, POLYPORALES) FROM BRAZILIAN CERRADO: AMAURODERMA CALCITUM SP. NOV. AND TAXONOMIC AND DISTRIBUTION NOTES ON OTHER SPECIES Studies in *Amauroderma* (Ganodermataceae, Polyporales) from Brazilian Cerrado: *Amauroderma calcitum* sp. nov. and taxonomic and distribution notes on other species

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#### Abstract

During a polypores survey in the Cerrado of "Parque Nacional da Chapada dos Guimarães", Mato Grosso State (Brazil), specimens of *Amauroderma* were collected and some of them deserved special taxonomic attention, for its intermediate morphology between *A. camerarium* and *A. omphalodes* and between *A. calcigenum* and *A. partitum*. Comparative study of the types and reference material led us to conclude that *A. camerarium* and *A. omphalodes* are synonyms, being *A. camerarium* the current name, and there is an undescribed species, *Amauroderma calcitum*, related to *A. calcigenum* and *A. partitum*. Moreover, *Amauroderma aurantiacum*, *A. brasiliense*, *A. schomburgkii* and *A. sprucei* are presented as new records for the Brazilian Cerrado and illustrations, taxonomic notes and discussion of each species, as well as a key of *Amaurodema* species with occurrence in Brazil are provided.

#### Introduction

Amauroderma Murrill (1905:366) comprises usually stiptate polypores species, with basidiopores globose to ellipsoid, without a truncate appex, double walled with the inner layer ornamented (rarely smooth), associated with dead wood or roots of living or dead trees. The most important taxonomical contributions to the genus were made by Torrend (1920), Ryvarden & Johansen (1980), Furtado (1981), Moncalvo & Ryvarden (1997) and Ryvarden (2004). In Brazil, recent
studies have been carried with a taxonomic treatment of some species (Coelho *et al.* 2007, Gibertoni *et al.* 2008, Campacci & Gugliotta 2009, Gomes-Silva *et al.* 2010, Gomes-Silva & Gibertoni 2012, Campos-Santana & Loguercio-Leite 2013). In Cerrado the knowledge about *Amauroderma* is restricted to checklists, and only four species [*Amauroderma calcigenum* (Berk. 1856:172) Torrend (1920:129), *A. omphalodes* (Berk. 1856:172) Torrend (1920:131), *A. praetervisum* (Pat. 1889:78) Torrend (1920:131) and *A. rude* (Berk.) Torrend (1920:127)] have been registered (Gibertoni & Drechsler-Santos 2010, Abrahão *et al.* 2012).

During the study of collections from Cerrado of Parque Nacional da Chapada dos Guimarães, some specimens seem to have interesting intermediate morphology between A. camerarium (Berk. 1856:143) J. Furtado (1968:140) and A. omphalodes, and between A. calcigenum (Berk.) Torrend and A. partitum (Berk. 1856:170) Wakef. (1934:242). Amauroderma camerarium and A. omphalodes were described in the same work by Berkeley (as Polyporus camerarius Berk. and Polyporus omphalodes Berk.) from Spruce's collection of the same type locality. Furtado (1981), in the genus review, pointed a great similarity between the taxa, being the main difference in the microstructures of pilear surface, a cortex in A. camerarium and crust in A. omphalodes. The taxonomic status of A. calcigenum and A. partitum was discussed several times in the literature, being considered as synonym by Furtado (1981) and Ryvarden (1984) and as distinct species by Ryvarden (2004) and Gomes-Silva et al. (2010), which considered the main difference the thickness of pileus and basidiopores size.

The detailed examination and comparison with reference material showed that *A. camerarium* and *A. omphalodes* are indeed synonyms and those specimens intermediate to *A. calcigenum* and *A. partitum* represent a new species, named here as *Amauroderma calcitum*. Additionally, other *Amauroderma* species from Cerrado domain are recorded for the first time, and taxonomic discussion and comments about distribution and/or morphology are presented.

### **Materials & Methods**

Samples were collected since 2011 to 2014 in the Parque Nacional da Chapada dos Guimarães (15°10'S-15°30'S e 55°45' W-56°00'W), Mato Grosso State, inserted in the Brazilian Cerrado. According to Köppen classification, the climate is Aw and Cw, both

characterized as hot and humid, with rainy (October to March) and dry (April to September) seasons (Pires & Mota 2009). The terms Amazonia, Atlantic Rain Forest, Caatinga and Cerrado are used here as phytogeographic domains. Samples were processed following Lodge *et al.* (2004). Macro and microscopically analyzes (n=40) of basidiomata follows Teixeira (1995) and Ryvarden (2004), abbreviations and color codes follows Amalfi *et al.* (2010) and Munsell (1975), respectively. Ultra-structural analyses (SEM) were realized in Laboratório Central de Microscopia Eletrônica (LCME-UFSC). Specimens studied are preserved at FLOR. Reference material from SP, BPI and K herbaria were used to morphological comparison. Herbarium acronyms follows Thiers B. [continuously updated].

## Results

A total of 51 specimens were collected and revised, representing seven species. *Amauroderma aurantiacum* [Torrend (in Bresadola 1932:1007)] Gibertoni & Bernicchia (2008:322), *A. brasiliense* (Singer 1983:22) Ryvarden (2004:44), *A. schomburgkii* (Mont. & Berk. 1844:331) Torrend (1920:140) and *A. sprucei* (Pat. 1894:75) Torrend (1920:121) are reported as new records for the Brazilian Cerrado , *A. omphalodes* is synonymized under *A. camerarium* and *Amauroderma calcitum* is presented as new due its intermediate morphology. *Amauroderma praetervisum* was already recorded for Cerrado and was also studied here. Illustrations, taxonomic notes and discussion of each species, as well as a key of *Amaurodema* species with occurrence in Brazil are provided.

## Taxonomy

Amauroderma calcitum Costa-Rezende & Drechsler-Santos sp. nov. (Fig. I a-f, II a-c).

Basidiomata pileate, thickened, stipitate, pilear surface slightly shine, reddish brown, concentrically zonated. Context pale, with or without one or two brown bands. Hymenophore with large circular to angular pores. Hyphal system dimitic, clamped generative hyphae, skeletal hyphae dextrinoid. Basidiospores ellipsoid.

Mycobank

**Type:**—BRAZIL. MATO GROSSO: Chapada dos Guimarães, Parque Nacional de Chapada dos Guimarães, 07 january 2013, *D.H. Costa-Rezende 105* (FLOR).

**Etymology:**—The name is in reference to the intermediate morphology of this taxa in comparison to *A. partitum* and *A. calcigenum*.

mesopodal, pleuropodal Basidiomata stipitate. or pseudomesopodal, single to occasionally with two fused pileus, unusually with superposed pileus on the upper surface (Fig. 1-e); pileus 25-70 mm in diameter, up to 6.0 mm thick, circular, subinfudibuliform or almost flattened to slightly convex, deeply umbilicate at center in younger specimens, coriaceous when fresh, coriaceous to corky when dry; margin acute to obtuse, regular and entire. Pilear surface slightly shine, dark reddish brown 2.5YR (3/4, 2.5/4) with very dusky red (2.5/2)concentric zonation, slightly sulcate, radially finely strigose (under the lens), glabrous. Context firm, almost white to pale vellow 2.5Y (8/4), with or without one or two brown lines coming from the stipe, 0.5-1 mm thick. Tubes concolor with context, up to 5 mm long. Pore surface concolor to slightly darker than the context in young specimens to pale brown 10YR (6/3) in fully developed basidiomata; pores circular and regular in young specimens to angular and irregular in older ones, well or not well-delimited, 1-2 (3) per mm, (400) 450-900 (1000) µm in diameter, (ave =  $657.9 \mu m$ ); *disseptiment* entire or strongly lacerate and almost irpicoid, (50) 60–180 (200)  $\mu$ m, (ave = 127.1  $\mu$ m). Stipe solid to tubular in some portions, straight to slightly tortuous, up to 100 mm long and 5 mm in diameter; surface velutinose (under the lens), brown 10YR (5/3); context concolor with pilear context, with two brown bands at least near the pileus; *cuticle* distinct brown. *Hyphal pegs* variably present,  $130 \times 20 \,\mu\text{m}$ .

*Pilear surface* composed by clamped generative hyphae,  $3-5 \mu m$  in diameter, thin to slightly thick walled intermixed with solid skeletal hyphae, both parallel to contextual hyphae. *Hyphal system* dimitic; *context* composed by clamped generative hyphae, (2)  $3-5 \mu m$  in diameter, hyaline, thin walled; and skeletal hyphae aciculiform or with two terminal branches,  $5-6 \mu m$  in diameter, up to 900  $\mu m$  without branches, hyaline to pale yellowish, straight or tortuous, thick walled to almost solid; *trama* of tubes composed by clamped generative hyphae,  $3-5 \mu m$  in diameter, hyaline, thin walled; and skeletal hyphae hyphae,  $3-5 \mu m$  in diameter, hyphae, thin walled; and skeletal hyphae hyphae,  $3-5 \mu m$  in diameter, hyphae, thin walled; and skeletal hyphae

aciculiform or with few apical branches (2-4) in variable size,  $4.5-6 \mu m$  in diameter, up to 700  $\mu m$  long (basal clamp non-observed), some of skeletal hyphae with few lateral aborted branches, both in the main stalk and branches, variably dextrinoid.

Basidia clavate,  $35-50 \times 15-30 \ \mu m$ .

Basidiospores ellipsoid, 11–14 (15) × (7) 8–10.5 (11) um, (ave =  $12.8 \times 9.4 \mu$ m), Q = 1.24–1.61, (ave-Q = 1.37), pale yellowish, double walled with the inner layer finely and regular ornamented, IKI-.

**Ecology and distribution:**—Growing always associated to thin roots on the ground. Frequently collected during the year in Cerrado of Parque Nacional de Chapada dos Guimarães—Mato Grosso state. Also collected in the Serra do Roncador (SP 102719), Barra do Garças—Mato Grosso state, probably in transition between Cerrado and Amazonia domain.

**Specimens examined:**— BRAZIL. Mato Grosso: Barra do Garças, Serra do Roncador, Trail R14, 31 May 1968, *D.M. Vital* (SP 102719); Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, sitio Vale do Rio Claro, 15 May 2011, *D.H. Costa-Rezende* 031 (FLOR); 07 January 2013, *D.H. Costa-Rezende* 104 (FLOR); *D.H. Costa-Rezende* 105 (FLOR); *D.H. Costa-Rezende* 106 (FLOR); *D.H. Costa-Rezende* 105 (FLOR); 12 January 2014, *L. Pereira-Silva 007* (FLOR); *L. Pereira-Silva 009* (FLOR); *L. Pereira-Silva 015* (FLOR); *L. Pereira-Silva 015* (FLOR); *L. Pereira-Silva 016* (FLOR); *L. Pereira-Silva 019* (FLOR); *L. Pereira-Silva 069* (FLOR); *L. Pereira-Silva 071* (FLOR); Sitio Véu da Noiva, 27 November 2012, *G. Alves-Silva 071* (FLOR); *G. Alves-Silva 072* (FLOR); *G. Alves-Silva 074* (FLOR); Primavera do Leste, near to Lagoa Vô Pedro Piana, 27 march 2013, *D.H. Costa-Rezende* 130; APP IMAmt, near to Córrego Alminhas, 22 december 2013, *G. Alves-Silva 549* (FLOR).

Additional specimens examined:—*Amauroderma calcigenum.* BRAZIL. sine datum, Gardner (BPI 237146, isotype); Goiás: Crixás, sine datum, A. Brookes (BPI 302396, type of A. brockesii); Bahia: sine datum, C. Torrend (BPI 307270, type of A. torrendii); Pernanbuco: Buíque, Parque Nacional do Catimbau, Paraíso Selvagem, 04 August 2006, E.R. Drechsler-Santos 034 (URM 80725); 28 May 2006, E.R. Drechsler-Santos et al. 013 (URM 80661); Caruaru, Estação Experimental do IPA, 13 September 2007, E.R. Drechsler-Santos 41 (URM 80634); Rondônia: Porto Velho, Estação Ecológica de Cuniã (ESEC), 13 September 2007, *Gomes-Silva et al. 2111* (URM 82777); ARGENTINA. Jujuy: Ledesma, Parque Nacional Calilegua, Mesada de las colmenas, Sendero La Cascada, 22 May 2007, *G. Robledo 1567* (CORD); Sendero Pedemontano, 02 April 2008, *Robledo 1875* (CORD). *Amauroderma partitum*. BRAZIL. Amazonas: Panuré, February 1853, *R. Spruce 200* (K 181861, type); Rondônia: Porto Velho, Parque Natural Municipal de Porto Velho, 03 february 2011, *A. C. Gomes-Silva et al. 2041* (URM 82872); Parque Natural Municipal de Porto Velho, 12 January 2009, *A.C. Gomes-Silva 726* (FLOR 4554); *A.C. Gomes-Silva 724* (FLOR 44756); PERU. Cusco: Quincemil, Vitobamba, 07 march 2010, *C.A. Salvador-Montoya 22* (FLOR 48461); Cerro de Pasco: Oxapampa, Palcazu, Paujil, 14 March 2009, *C.A. Salvador-Montoya 296* (FLOR).

**Remarks:**—*Amauroderma calcitum* is characterized by its robust basidiome with a reddish brown, slightly shine pilear surface, in section with a thin cuticle, a pale context with or without brown bands, a brown, robust (in mature specimens) and slightly velutinose stipe, up to 5 mm in diameter, large pores with lacerate dissepiments in mature specimens, a dimitic hyphal system with skeletal hyphae variably dextrinoid and ellipsoid, pale yellowish, double-walled basidiospores, with the inner layer finely and regular ornamented.

The morphologically closest related species are A. calcigenum (Fig I g, II d-e) and A. partitum (Fig I h-k, II f-g), mainly due to the color of pilear surface and shape and size of pores and basidiospores. The taxonomic status of these two taxa has been discussed several times, Furtado (1981) and Ryvarden (1984) treated A. calcigenum and A. partitum as synonyms, the former also included other taxa into the synonym [Stereum miquelianum Mont (1851:203), Hexagonia gracilis Berk. (1858:237), Polyporus brockesii Lloyd (1912:5), Polyporus torrendii Lloyd (1915:4), and all its homotypic synonyms]. Later, Ryvarden (2004) suggested that the more robust pileus and the larger basidiospores of A. calcigenum segregate these taxa. Gomes-Silva et al. (2010), agreeing with Ryvarden (2004), observed that A. calcigenum presents basidiomes with thick pilei (up to 12 mm), while A. partitum has smaller and more fragile basidiomes, with thinner stipe and pileus, that is usually pliable. Microscopically the former presents slightly longer and distinctly wider basidiospores. The same author also observed that both taxa show a variable presence of hyphal pegs and considered it as not valuable taxonomic character in this case.

Considering pilear thickness and basidiospores size. Amauroderma calcitum presents an intermediate morphology between A. calcigenum e A. partitum, i.e., the robust basidiomata is similar as in A. calcigenum and the size and shape of spores are similar as in A. partitum (Table I). The overlap or intermediate features suggests these taxa could be interpreted as synonyms; however the detailed morphological study of A. calcitum, A. calcigenum and A. partitum, including type collections and reference material, led us to conclude that there are three distinct species. Amauroderma calcitum presents a more robust basidiomata than A. partitum, both in pileus (up to 6 mm vs 1.1 mm thick) and stipe (up to 5 mm vs 1,5 mm thick), larger pores [(400) 450-910 (1000) vs (300) 350-650 (1000) µm in diameter] and a larger average value of the basidiospores size  $(12.8 \times 9.4 \text{ vs } 11.6 \times 8.9 \text{ \mum})$ . Some young specimens of A. calcitum seem similar to A. partitum, nonetheless, these specimens are not fertile yet, with clearly non developed tubes and pores.

Although the thickness of the pileus is a representative feature to segregate *A. calcitum* and *A. partitum*, this character is not useful to segregate *A. calcitum* and *A. calcigenum*, because both species presents thicker pileus. On the other hand, *A. calcigenum* presents a softer pileus, darker context and larger (wider) basidiospores than *A. calcitum* [(12) 13.5–16 × 10–12 (12.5) *vs* 11–14 (15) × 8–10 (11) µm]. Type specimens of *A. brockesii* and *A. torrendii* also present larger basidiospores, with a similar range of *A. calcigenum*. Unfortunately, we could revise just a small fragment of the type specimen of *A. calcigenum*, so we cannot discuss its status against the synonyms *A. brockesii* and *A. torrendii*.

The specimen SP 102719, considered by Furtado (1981) as *A. calcigenum*, is treated here as *A. calcitum*. It is possible that specimens with its intermediate morphology lead the author to synonymize *A. calcigenum* and *A. partitum*, similar criterion was used by him to synonymize *Fomes paulensis* Henn (1904:202) in *A. sprucei*.

Amauroderma camerarium (Berk.) J. Furtado, Rev. Gên. Amauroderma (Polyp.). Est. Bas. Microestr. Basid. 140. 1968 (Fig. III aj, IV a-m)

≡ Polyporus camerarius Berk., J. Bot. & Kew Misc. 8: 143. 1856.

*≡ Fomes camerarius* (Berk.) Sacc., Syll. fung. (Abellini) 6: 153. 1888.

*= Polyporus omphalodes* Berk., Hooker's J. Bot. Kew Gard. Misc. 8: 172. 1856.

 $\equiv$  Fomes omphalodes (Berk.) Sacc., Syll. fung. (Abellini) 6: 153. 1888.

 $\equiv$  Ganoderma omphalodes (Berk.) Pat., Bull. Soc. mycol. Fr. 5(2,3): 77. 1889.

 $\equiv$  Scindalma omphalodes (Berk.) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 519. 1898.

 $\equiv$  Amauroderma omphalodes (Berk.) Torrend, Brotéria, sér. bot. 18: 131. 1920.

Basidiomata stipitate, pleuropodal to pseudomesopodal; pileus 20-40 mm in diameter, 3-7 mm thick, sub-woody to wood when dried, single, conchate to reniform, slightly concave, in section with or without a thin dark cuticle, coriaceous when fresh, margin sub-acute to obtuse, regular to undulate. Pilear surface dull, very pale brown 10YR (8/4) with concentric dark reddish brown 5YR (3/2, 3/3, 3/4) zones, to very dark gray 10YR (3/1) or dark reddish brown 5YR (3/2) with inconspicuous concentric zones, slightly sulcate, glabrous. Context firm, almost white to pale yellow 2.5Y (8/4), with one or two dark bands from the stipe, 1-3 mm thick,. Tubes slightly darker than context, 2-8 mm long. Pore surface light gray (7/2) to light brownish gray (6/2); pores regular, (3) 4-7/mm, (80) 110–210 (230) µm in diameter, (ave = 147.2  $\mu$ m); dissipments entire, 50–190 (230)  $\mu$ m, (ave = 100.4  $\mu$ m). Stipe solid to tubular, straight or tortuous, yellowish brown 10YR (5/4), context of stipe concolor with pileus, with two dark bands; *cuticle* dark brown, up to 120 mm long.

*Pilear surface* as an indeterminate derm, or cortex or crust. *Hyphal system* dimitic: *context* composed by clamped generative hyphae, 2.0–3.0 µm in diameter, hyaline, thin walled; skeleto-binding hyphae, up to 6 µm in diameter, thin to thick walled, formed by a main stalk and very short lateral branches, with or without two thin apical branches, pale yellow; typical arboriform skeletal hyphae, up to 6 µm in diameter, with straight or tortuous main stalk, thick-walled to almost solid, with short or long branches, pale yellow; *trama of tubes* composed by clamped generative hyphae, 2.0–5.0 µm in diameter, hyaline, thin walled; typical arboriform skeletal hyphae, up to 5.0 µm in diameter, with short or long branches, with straight or tortuous main stalk, thick walled to almost solid, pale yellow. *Basidia* not observed. *Basidiospores* subglobose to broadly ellipsoid, (10) 11–14 (15) × (8) 9–12 (13) µm,

(ave =  $12.5 \times 10.6 \ \mu$ m), Q = (1.00) 1.08–1.30, (ave-Q = 1.18), thick walled, with conspicuous endosporic projections, yellowish, variable dextrinoid when immature.

**Distribution:**—South Brazil to Belize. In Brazil this species has been recorded for Amazonia, Atlantic Forest, Caatinga and Cerrado (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Gugliotta *et al.* 2014).

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Véu de Noiva, 19 February 2012, *G. Alves-Silva s/n* (FLOR); 19 February 2012, *G. Alves-Silva 90* (FLOR); 19 February 2012, *G. Alves-Silva 131* (FLOR); 20 May 2012, *G. Alves-Silva 171* (FLOR); 19 February 2012, *G. Alves-Silva 172* (FLOR); 19 February 2012, *G. Alves-Silva 172* (FLOR); 19 February 2012, *G. Alves-Silva 173* (FLOR); 01 May 2013, *G. Alves-Silva 475* (FLOR); Sítio Vale do Rio Claro, 07 January 2013, *D.H. Costa-Rezende 112* (FLOR).

Additional specimens examined:—BRAZIL. Amazonas: Panuré, March 1853, Spruce 171 (BPI 327148, syntype of *A. camerarium*); Spruce 32 (NY 730851, type of *A. omphalodes*); Mato Grosso: Aripuanã, Cachoeira Dois Irmãos, 06 July 1962, M. Mee (SP 61451, *A. camerarium*).

**Remarks:**—*Amauroderma camerarium* is characterized by its stipitate, dull, very pale brown to dark reddish brown pileus, a pale context with dark bands from the stipe and pale yellow, thick-walled and large spores with conspicuous ornamentation.

The type specimens of *A. camerarium (Polyporus camerarius*, Spruce 171 and 197) and of *A. omphalodes (Polyporus omphalodes*, Spruce 32 and 194, Fig. III a) were collected in Panuré (Brazilian Amazonia), and both were described by Berkley (1856). Berkley has not presented a morphological comparison between these taxa, when we compare the protologues is not possible to detect a substantial difference between them. Furtado (1981), when taxonomically revised the genus, pointed out that the distinction of those species is placed in the microstructures of pilear surface of the taxa, *i.e.*, *A. camerarium* presents a cortex, while *A. omphalodes* a crust. The cottony consistence of *A. omphalodes* when torn was recorded by the author as well. On the other hand, Ryvarden (2004) considered the presence of a dextrinoid reaction and a paler pilear surface for *A. camerarium* and one or two black lines in context for *A. omphalodes* as main differences. However, the author mentioned for both species a pilear surface composed by agglutinated hyphae without any distinct structure (some attributes of Furtado's and Ryvarden's sense is represented in Table II). It is important to note that Furtado (1981) did not study Melzer's reaction neither the presence of lines in the context.

The type specimen of *A. omphalodes* (Spruce 32) is well preserved, and in the pilear surface a crust with strongly thickened generative hyphae was easily observed. In contrast, we just received a small piece of the type of *A. camerarium* and the microstructures of pilear surface and spores were not possible to observe. The specimen SP 61541 (Fig. III b), identified by Furtado (1981) as *A. camerarium*, was also in a well preserved and presents a cortex in the pilear surface. Among our collections, three specimens [GAS s/n (Fig. IV f), GAS 171, GAS 172] present a cortex, such as in SP 61541(Fig. IV b). Other five specimens were observed (Table II), two of them present an indeterminate derm [GAS 90, GAS 131 (Fig. IV c,d)] and the rest (DHCR 112, GAS 198, GAS 475) present a young crust, with clamped generative hyphae, slightly thick-walled, apparently without the wall deposition that Furtado (1981)called inscrustations.

Besides the significant macromorphological similarities, as already observed by Furtado (1981) and Ryvarden (2004), the reference material and our collections present similar basidiospores, both in size and shape (Table II, Fig. IV). Additionally, all specimens revised present pale yellowish, thick-walled and conspicuous ornamented spores. On the other hand the dark bands in the context of stipe and pileus were observed in all materials studied by us, both *A. camerarium* and *A. omphalodes* specimens. The type specimen of *A. omphalodes* presents strong dextrinoid skeletal hyphae, while the reference collections of *A. camerarium* and ours are variable, being negative (IKI-), or weak dextrinoid, mainly when observed in masses.

The morphological comparison among the type, reference specimens of *A. camerarium* and *A. omphalodes* and our collections led us to consider that there are no significant differences between those taxa. Furtado (1981) pointed out that an ontogenetic variation occurs in the microstructures of pilear surface of *A. omphalodes* and we conclude that the cortex could be a growth variability and/or a feature influenced by weathering. Moreover, the presence of dark bands in stipe and context, the variable IKI skeletal hyphae and the similar shape and size

of basidiospores are also evidences that *A. omphalodes* and *A. camerarium* are the same species. *Amauroderma camerarium* was described in the same work of *A. omphalodes*, having the former priority due to the number of pages.

Regardin other Neotropical species, *A. praetervisum* (Pat.) Torrend and *A. pseudoboletum* (Speg. 1883:16) J.S. Furtado (1968, 230) also present black bands in the context and large spores; however the paler, thinner walls and less conspicuous endosporic ornamentation of spores from the former are distinctive features. Furthermore, *A. praetervisum* presents a darker pilear surface. *Amauroderma pseudoboletum* (Speg.) J.S. Furtado presents larger pores, 3-5/mm [(210) 250–350 (400) µm in diameter]. *Amauroderma sprucei* also presents a pale context and small pores, being different mainly due its orange pore surface in most of specimens, absence of contextual black bands and smaller spores [(7) 8–10 × 7–9 µm].

# Taxonomic notes on other Amauroderma species that occur in Cerrado

Amauroderma aurantiacum (Torrend) Gibertoni & Bernicchia (Fig. V a, b, h)

Description:—in Furtado (1981) and Ryvarden (2004).

**Distribution:**—Brazil and Venezuela. In Brazil this species has been recorded for Amazonia and Atlantic Forest. The type specimen is indicated as from Goias states, but the specific locality is unknown (Ryvarden & Iturriaga 2001, Ryvarden 2004, as *A. macrosporum*, Gibertoni *et al.* 2008, Campacci & Gugliotta 2009, Gugliotta *et al.*, 2014). In this case, this is the first formal report of species for the Cerrado domain.

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 15 May 2011, *D.H. Costa-Rezende 30* (FLOR); 12 January 2014, *L. Pereira-Silva 02* (FLOR); L. *Pereira-Silva 03* (FLOR); *L. Pereira-Silva 04* (FLOR); *L. Pereira-Silva 08* (FLOR); *L. Pereira-Silva 12* (FLOR); *L. Pereira-Silva 14* (FLOR); *L. Pereira-Silva 17* (FLOR); *L. Pereira-Silva 25* (FLOR); Sitio Véu de Noiva, 19 Janaury 2012, *G. Alves-Silva 77* (FLOR).

Remarks:-The thick and soft pileus when fresh, orange to reddish brown pilear surface, large pores (1-2/mm) and globose to subglobose (13) 14–17 (18)  $\times$  13–17 (18) µm, yellowish, thick-walled spores, with conspicuous endosporic projections in sub-reticulate pattern, are distinctive to this species. The presence of trichodermal structures in the pilear surface were not reported by Furtado (1981) and Ryvarden (2004), probably due to the specimens revised were in an old condition and this feature was not possible to see, as observed by us in old specimens. However, we have observed a trichodermal structures in young specimens, composed by loose generative hyphae, up to 120 µm long, with or without clamps and adventitious septa. These generative hyphae are founded projecting from a layer formed by moderately thickwalled generative hyphae, parallel to the context hyphae. For this species, the hyphal system was described differently in the literature. Furtado [1981, as A. macrosporum Furtado (1968:203)] considered as trimitic, composed by clamped generative hyphae, branched and thickened binding hyphae, with thinner sub-solid branches, and arboriform skeletal hyphae.Ryvarden (2004, as A. macrosporum) described as dimitic, composed by clamped generative and arboriform skeletal hyphae. In our study, we observed skeleto-binding hyphae formed by a main stalk and very short lateral branches, with or without two thin apical branches, and typical arboriform skeletal hyphae in the context and only arboriform skeletal hyphae in the trama of tubes.

Amauroderma brasiliense (Singer) Ryvarden (Fig. V c, i, j)

Description:—Ryvarden (2004) and Coelho et al. (2007).

**Distribution:**—Brazil and Venezuela. In Brazil has been recorded for Amazonia and Atlantic Forest biomes (Ryvarden 2004, Coelho *et al.* 2007, Campacci & Gugliotta 2009, Gugliotta *et al.* 2014), being here reported as first for Cerrado domain.

**Specimens examined:**—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Véu da Noiva, 26 March 2013, D.H. Costa-Rezende 128 (FLOR).

Additional specimen examined:—BRAZIL. São Paulo: Ilha Cardoso, February 1987, [SP 213543, type of *Amauroderma corneri* Gulaid & Ryvarden (1998:28)]

Remarks:—The soft basidioma when fresh, the pale context, dominated by clamped generative hyphae and globose to ellipsoid spores [(6)  $7-10 \times (5.5)$  6–8 (9) µm] characterize the species. Singer (1983, as Scutiger brasilienses Sing.) considered the hyphal system as monomitic and Ryvarden (2004) described as dimitic, with skeletal hyphae scattered in basidiome, some of that with swollen apex (clamidospore?). On the other hand, Coelho et al. (2007) considered monomitic to probably dimitic, with long and thick-walled contextual hyphae sometimes resembling skeletal hyphae, but with a basal clamp. Furthermore, the authors observed dendrohyphidia, gloeoporus hyphae and gloeocystidia, which none of these structures were observed by us. Additionally, for us, the context is composed only by clamped generative hyphae, thin to slightly thick walled, some distinctly wider, with a swollen apex, up to 30 µm wide. Probably it was interpreted as gloeoporus hyphae by Coelho et al. (2007) and as skeletal with swollen apex by Ryvarden (2004). Finally, we define the trama of tubes construction as dimitic, composed by clamped generative and arboriform skeletal hyphae. Ryvarden (2004), though not mentioned in the description, probably observed the arboriform skeletal hyphae as it is possible to see in the plate of this species (figure 12 of his book).

In fact, despite the differences presented in the literature, the hyphal system of *A. brasiliense* is greatly particular and deserves special taxonomic attention. The monomitic contex, dominated only by generative hyphae, is unique in the genus. A detailed revision of several specimens, previously determined as *A. brasiliense*, and a phylogenetic analyses should be done in order to delimit the taxon, broadening the *Amauroderma* circumscription of *Amauroderma* or finding another genus in which the species could fits in a natural classification.

Amauroderma praetervisum (Pat.) Torrend (Fig. V d, e, k, l)

Description:—in Furtado (1981) and Ryvarden (2004).

**Distribution:**—Brazil, Colombia, Costa Rica, Panama and Mexico. In Brazil this species has been recorded for Amazonia, Atlantic

Rain Forest, Caatinga and Cerrado (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Abrahão *et al.* 2012, Gugliotta *et al.* 2014).

**Specimens examined:**—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 12 January 2014, L. Pereira-Silva 013 (FLOR); 12 January 2014, L. Pereira-Silva 066 (FLOR); 12 January 2014, L. Pereira-Silva 067 (FLOR); 12 January 2014, L. Pereira-Silva 068 (FLOR).

Additional specimen examined:— Amauroderma praetervisum. BRAZIL. Sine datum, Weddel (BPI 237178, isotype); COLOMBIA. La Jagua, Magdalena Valley, 07 September 1924, A. Cyril 610 (BPI 237179). Puerto Lopez, March 1945, L. Ruby 10355 (BPI 237193); COSTA RICA. Limon Province: 10.12.1929, C.W. Dodge (BPI 747518); Amauroderma pseudoboletum.São Paulo: Cantareira, Horto Florestal, 28 March 1962, J.S. Furtado (SP 61135).

**Remarks:**—The pale context with two black bands and a thin dark cuticle, small pores (4–5/mm) and distinct core in the stipe, which is paler and softer than outside layer, characterize macroscopically the species. Microscopically, it is recognized by a crust composed by some free hyphae in the pilear surface, trama of tubes with variable dextrinoid skeletal hyphae and large broadly ellipsoid spores [(11.5) 12–13.5 (14)  $\times$  10–12 (13) µm].

The dimitic hyphal system, composed by clamped generative and arboriform skeletal hyphae, was already observed by Ryvarden (2004). On the other hand, Furtado (1981) pointed out a trimitic hyphal system, composed by clamped generative, binding much branched, and by arboriform and aciculiform skeletal hyphae. As not reported before, we observe a dextrinoid reaction in skeletal hyphae.

Regarding the pale context, consistence of pileus and size of pores, *A. camerarium* and *A. sprucei* could be related. *Amauroderma camerarium* was treated above and *A. sprucei* presents a whitish context and smaller basidiospores [(7.0)  $8.0-10 \times 7-9 \mu$ m], as well as an orange pore surface in many specimens. *Amaurodema schomburgkii* also presents a robust pileus, with similar consistence, black bands in the context and small pores, being different due to darker context, smaller spores and a cortex in the pilear surface. *Amauroderma pseudoboletum* presents a context with similar color and similar size of spores, being different due to spores with more conspicuous ornamentation.

Amauroderma schomburgkii (Mont. & Berk.) Torrend (Fig. V f,

m, n)

Description:—in Furtado (1981) and Ryvarden (2004).

**Distribution:**—This seems to be the most common *Amauroderma* species in the Neotropics, occurring since South Brazil to Central America. In Brazil, has been recorded for Amazonia and Atlantic Forest (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Gugliotta *et al.* 2014). In Mato Grosso states, was recorded for Serra do Roncador by Furtado (1981), being not possible to determine if it is from Cerrado or Amazon domain (Gibertoni & Drechsler-Santos 2010, Abrahão *et al.* 2012). Here we present the first formal report of species for Cerrado.

**Specimens examined:**—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Véu da Noiva, 10 March 2013, G. Alves-Silva 393 (FLOR); 10 March 2013, G. Alves-Silva 401 (FLOR).

Additional specimen examined:—*Amauroderma schomburgkii.* BRAZIL. Amazonas: Panuré, Spruce 192 [BPI 215326, isotype of *P. ocellatus* Berk. (1856:172)]; FRENCH GUIANA. Leschenault [BPI 237166, isotype *A. heteromorphum* (Lév.) Torrend (1920:139)]; Track to Montage Tortue, Route de Belizon, 15 km from road N2, C. Feuillet 3293 (BPI 747459); COSTA RICA. Cerro Guaca, Quebrada de Laja and Rio Nuevo, 03 March 1930, C.W, Dodge (BPI 747390). PANAMA. Rio Indio Hydrographic Station and the Natural Bridge of Rio Puente, 13 January 1935, C.W. Dodge (BPI 237122). COSTA RICA. Puntarenas, near Rio Sandoval, 25 April 1939, C.W. Dodge (BPI 237192, 237192); *Amauroderma exile*. BRAZIL. *sine datum*, R. Spruce (K 180667, isotype); Rondônia: Porto Velho, Parque Natural Municipal de Porto Velho, 03 February 2011, Gomes-Silva *et al.* (URM 83411).

**Remarks:**—This species is recognized by its golden brown context, with dark bands, cortex pilear surface, small pores (5-7/mm) and globose to sub-globose basidiospores [(8)  $9-12 \times (7) 9-11 \mu \text{m}$ ].

In our observations, the context is composed by clamped generative, long (up to 1500  $\mu$ m long) unbranched skeletal, and thin to thick walled skeleto-binding hyphae, formed by a main stalk and short

lateral branches, with or without two thin apical branches. In the trama of tubes, the hyphal system is composed by clamped generative and short, clavate (up to 170  $\mu$ m long in main stalk, 2.5  $\mu$ m in diameter at the basis to 11  $\mu$ m at the apex) arboriform skeletal hyphae, with short branches, variable ramified, thick walled to solid, the first usually with several adventiceous septa. On the other hand, Furtado (1981) and Ryvarden (2004) treated the hyphal system as distinct: as trimitic, composed by clamped generative, binding, and arboriform and aciculiform skeletal hyphae; and as dimitic, composed by clamped generative and arboriform skeletal hyphae, respectively. Except for the slightly larger spores of some specimens studied than those described by Furtado (1981) and Ryvarden (2004) [7–9 (11)  $\mu$ m and 7–10  $\mu$ m, respectively], all the other features are according to the *A. schomburgkii* circumscription.

Amauroderma exile (Berk. 1856:173) Torrend (1920:142) and A. sprucei also present small pores and similar shape of basidiopores. However, A. exile presents a shine reddish brown and flexible pileus, distinct of A. schomburgkii, which usually presents a dull and hard basidiomes. Amauroderma sprucei is easily distinguished due to their whitish context, vivid orange pore surface, dextrinoid skeletal hyphae and by a crust in pilear surface.

Amauroderma sprucei (Pat.) Torrend (Fig. V g, o, p)

Description:—in Decock & Herrera-Figueroa (2006).

**Distribution:**—Brazil, Venezuela, French Guyana, Costa Rica, Belize and Cuba. In Brazil this species has been recorded for Amazonia, Atlantic Forest and Caatinga (Furtado 1981, Decock & Herrera-Figueroa 2006, Campacci & Gugliotta 2009, Drechsler-Santos *et al.* 2013, Gugliotta *et al.* 2014). Then, here is reported for the first time the occurrence of the species in the Cerrado domain.

**Specimens examined:**—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 07 January 2013, D.H. Costa-Rezende 113 (FLOR); 07 January 2013, D.H. Costa-Rezende 114 (FLOR); 07 January 2013, D.H. Costa-Rezende 115 (FLOR); 12 January 2014, L. Pereira-Silva 21 (FLOR); 12 January 2014, Pereira-Silva 22 (FLOR); 12 January 2014,

L. Pereira-Silva 58 (FLOR); 12 January 2014, L. Pereira-Silva 77 (FLOR); 12 January 2014, L. Pereira-Silva 79 (FLOR).

Additional specimens examined:—BRAZIL. Amazonas: Panure, Spruce 44 (BPI 237203, isotype of *A. sprucei*).

**Remarks:**—The whitish context, a vivid orange pore surface in some specimens, small pores [5–7 (8) pores/mm], a crust with a short trichoderm in the pilear surface, the strongly dextrinoid skeletal hyphae and predominantly subglobose spores [(7.0)  $8.0-10 \times 7-9 \mu$ m], with conspicuous columnar ornamentation, are characteristic of this species. Decock & Herrera-Figueroa (2006) described the hyphal system as ditrimitic, with generative and arboriform skeletal hyphae in the trama of tubes, without any comments about the hyphal composition of context. In our observations, the context is composed by clamped generative, long and tortuous skeletal (up to 7.0  $\mu$ m in diameter), with few apical branches, and intercalary skeleto-biding hyphae, with long lateral and apical ramified branches. In the trama of tubes, besides the generative and arboriform skeletal hyphae, we observed thick-walled skeleto-binding, formed by a main stalk and very short lateral branches, with or without two thin apical branches.

Regarding the pale context, consistence of pileus and size of pores, *A. camerarium* and *A. praetervisum* are morphologically related and the taxa were treated above. Despite of few differences in the description of hyphal system, we agree with morphological concept proposed by Decock & Herrera-Figueroa (2006).

## Key to Amauroderma species that occurs in Brazil

1Hymenophore with up to 4 pores/mm...2

- Hymenophore with more than 4 pores/mm...10

2 Pilear surface laccate ... Amauroderma renidens (Bres.) Torrend

- Pilear surface non-laccate...3

3 Basidiospores globose to subglose...4

- Basidiospores ellipsoid...8

4 Basidiospores with a semi-reticulate ornamentation pattern, 13–17 (18)  $\mu$ m in wide ... A. aurantiacum

4 Basidiospores with a columnar ornamentation pattern, up to 11  $\mu m$  in wide...5

5 Context monomitic...A. brasiliense

- Context dimitic...6

6 Pilear surface composed by swollen ventricose apical structures... A. boleticeum (Pat. & Gaillard) Torrend

- Pilear surface trichodermal...7

7 Pilear surface hirsute, basidiospores up to 8  $\mu$ m long... A. *trichodermatum* Furtado

- Pilear surface finely velutinose, basidiospores longer... A. *intermedium* (Bres. & Pat.) Torrend

8 Basidiomata thin and pliable, pileus thin (up to 2 mm)  $\dots$  A. partitum

- Basidiomata robust, pileus thicker (if thinner, the specimen is immature)... 9

9 Basidiospores up to  $15\times11~\mu m$  (average size  $12.8\times9.4)$  ... A. calcitum

- Basidiospores slightly larger (average size 14.1  $\times$  10.9)  $\ldots$  A. calcigenum

10 Stipe laccate... *A. picipes* Torrend - Stipe dull...11

11 Basidiospores oblong... A. oblongisporum Furtado

- Basidiospores globose to ellipsoid... 12

12 Basidiospores smooth... A. coltricioides Hankel, Aime & Ryvarden

- Basidiopores with the inner layer ornamented ...13

13 Basidiospores up to 11 µm wide ...14

- Basidiospores wider... 19

14 Basidiospores distinctly ellipsoid... A. elegantissimum Ryvarden & Iturr.

- Basidiospores globose to sub-globose... 15

15 Pilear surface as a cortex...16

- Pilear surface different (crust, paraderm or palisadoderm) ...17

16 Basidiomata usually with a shine reddish brown pilear surface and flexible pileus... A. exile

- Basidiomata with a dull dark to grayish brown pilear surface, usually hard (at least when fully developed)... A. schomburgkii

17 Context whitish to pale yellow, pore surface almost white to orange, pilear surface as a trichoderm... A. sprucei

- Context in different shades of brown, pore surface dark coloured, pilear surface different (paraderm or palisadoderm)... 18

18 Pilear surface as a paraderm (a short himeniderm in young specimens), basidiopores (7.5)  $9-11 \times (6) 8-10 \mu m$ , thin walled... A. subrugosum (Bres. & Pat.) Torrend

- Pilear surface as a crust derived from a palisadoderm, basidiospores 11–12  $\times$  9–10  $\mu m,$  thick walled... A. fasciculatum (Pat.) Torrend

19 Pileus up to 1 cm in diameter, skeletal hyphae IKI-, basidiospores  $15-17 \times 13-15 \ \mu m... A.$  unilaterum (Lloyd) Ryvarden

- Pileus more than 1 cm in diameter, skeletal hyphae variable dextrinoid, basidiospores up to  $15\times13~\mu m\dots\,20$ 

20 Basidiospores thin to slightly thick walled, finely ornamented... A. praetervisum

- Basidiospores thick walled, conspicuously ornamented... 21

21 Hymenophore with 4–7 pores/mm [(80) 110–210 (230)  $\mu m$  in diameter]... A. camerarium

- Hymenophore with 3-5 pores/mm [(210) 250–350 (400)  $\mu m$  in diameter]... A. pseudoboletum

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		Species		
Taxa and localities	Pores diam.	Basidiospores (µm)	Basidiospores average (µm)	Pileus thick. (mm )
A. calcitum				
Brazil. Mato Grosso (CR 105)	500-750 (800)	$11 - 13.5 \times 8 - 10$	$12.4 \times 9.25$	5
Brazil. Mato Grosso (CR 111)	450–800 (550) 580–910	11–14 × 9–10 (11)	12.6 × 9.5	5
Brazil. Mato Grosso (GAS 71)	(1000) (400) 450–905	11–14 × (8) 8.5 – 10 (10.5) (12) 12.5–14.5 (15) × 9–10.5	12.5 × 9.2	4
Brazil. Mato Grosso (GAS 72) Brazil. Mato Grosso (SP	(1000) (400) 495–802.5	(11)	13.4 × 9.85	3.5
102719)	(900)	12–14 × (8) 9–10	$13.2 \times 9.5$	5.5
Brazil. Mato Grosso (CR130)	(500) 550–750 (800)	(11) 12–14 (15) × 8.5–10 (11) 11.5–13 (14) × (7) 8–10	$12.8 \times 9.25$	2.5
Brazil. Mato Grosso (LPS 07)	*	(11)	$12.2 \times 8.9$	3.5
Brazil. Mato Grosso (LPS 69)	* (400) 450–800-	11–14 × (8) 8.5–10	12.3 × 9.2 12.2–13.4 x 8.9–9.85	6
	(1000)	11–14.5 (15) × (8) 9–10.5 (11)	$12.8 \times 9.4$	5
A. partitum	(300) 350–600	(10) 10.5–12 (13) × (7) 8–9.5		
Peru. (CS 22)	(1000)	(10)	$11.3 \times 8.5$	1.1

Table I. Macro and microscopic features of Amauroderma calcitum and related taxa.

Peru. (CS 294)	(300) 347–652 (700)	(10.5) 11–13 × 8–10 (10.5)	$12 \times 9.5$	1
Brazil. Rondônia (URM 82872) Brazil. Rondonia (FLOR	300-552 (600)	10–11.5 (12) × (7) 7.5 – 9	$10.8 \times 8.4$	1
44756)	(300) 395–700	11–13 (13.5) × (8) 8.5–10	$12 \times 9.1$	1
Brazil. Rondonia (FLOR 45554) Brazil Panuré (K 180861	300–450	11–13 × (7.5) × 8–10 (10.5)	12.3 × 8.9	0.9
syntipe)	(300) 350–570 (600)	*	*	1
	300-700 (1000)	10–13 × (7) 8–10 (10,5)	10.8–12.3 × 8.4–9.5 11.6 × 8.9	1
A. calcigenum				
Brazil. Pernanbuco (URM				
80725)	400-600	$(12) \ 13-15.5 \times \ (10) \ 10.5 - 12$	$14.5 \times 11.2$	6.1
Brazil. Pernanbuco (URM				• •
80661)	350-500 (650)	$13-16 \times 10-12$	$14.7 \times 11.1$	2.8
80634)	*	(13) 14–15.5 (16) $\times$ (10) 10.5– 12	$14.6 \times 11.1$	6
Brazil. Rondonia (URM 82777)	*	(11.5) 12–13.5 (14) × 10–11	$12.3 \times 10.3$	5
Argentina. Jujuy (Robledo 1567)	*	12–15 (16) × 10–11 (11.5)	$13.6 \times 10.7$	7
Argentina. Salta (Robledo 1875)	*	13–15 (16) × (9.5) 10–12	$14.4 \times 10.9$	4
Brazil. Goiás (BPI 237146				
Tipo)	*	(13) 13.5–16 × 10–12 (12.5)	$14.8 \times 11.1$	*
	350-500 (650)	(11.5) 13-16 × 10-12 (12.5)	$12.3-14.8 \times 10.7-11.2$	5,5

			14.1 × 10.9	
<b>A. brockesii</b> Brazil. Goiás (BPI 302396 Tipo)	350-700	(13.5) 14–16 (16.5) × 10–12 (12.5)	15×11.2	5
<i>A. torrendii</i> Brazil. Bahia (BPI 307270 Tipo)	*	(12) 13–15 × (9) 10–12 (12.5)	$13.7 \times 10.9$	9

\* The feature was not observed.

60

Table II. Pores and basidiospores size of Amauroderma omphalodes and Amauroderma camerarium, showing no relevant
differences and common overlap measures.

	Pores			Basidiospores size (µm)		
	A		ve Pores/m			Pilear
	Diam. (µm)	(µm)	m	Range	Ave	surface
P. omphalodes (NY 730851				12.0-14.0 (15) × 11.0-13.0	$13.4 \times$	
type)	(90) 100-130	114.5	4–6	(13.5)	12.0	Crust
A. omphalodes sensu						
Furtado (1981)	40-160	*	4–6	$11 - 13 \times 9 - 11$	*	Crust
A. omphalodes sensu						
Ryvarden (2004)	*	*	4–6	$11-14 \times 10-13$	*	-
				$11.0-13.0 \times (10) \ 10.5-12.5$	$12.2 \times$	
A. camerarium (SP 61541)	120.0-160.0 (170)	142.8	4–6	(13.0)	11.3	Cortex
A. camerarium sensu						
Furtado (1981)	70.0-100	*	5–7	$10.0-13.0 \times 9.0-11.0$	*	Cortex
A. camerarium sensu						
Ryvarden (2004)	*	*	5–7	12.0–15.0 (16.0) × 10–13	*	_
				$(10.0)\ 11.0-14.0 \times (9.0)$	$12.2 \times$	
A. camerarium (GAS 090)	80.0-150.0	119.5	(5) 6–7	10.0-12.0	10.7	Derm
				(10.0) 11.0–14.0 (15.0) ×	$12.4 \times$	
A. camerarium (GAS 131)	110.0-140.0 (160.0)	124.5	5-7 (8)	9.0-12.0	10.6	Derm
	(140.0) 150.0-210.0		(3.0)	(11.0) 12.0–14.0 × 9.0–11.0	$12.6 \times$	
A. camerarium (GAS 171)	(220.0)	179.3	4.0-5.0	(12.0)	10.2	Cortex
	(110) 130.0-200.0			12.0–13.5 (14.0) × 10.0–	$12.9 \times$	
A. camerarium (GAS 172)	(210.0)	161.0	4–6	12.0	11.1	Cortex
				12.0–14.0 (15.0) × 10.0–	$13.2 \times$	
A. camerarium (GAS 475)	100.0-120.0	114.0	5–6	12.0 (12.5)	11.0	Crust

	(120.0) 130.0–160.0					
A. camerarium (DHCR 112)	(170.0)	148,5	5–6	*	*	Crust
	(130.0) 140.0–180.0			(11.0) 12.0–14.0 × 10.0–	$12.7 \times$	Derm/
A. camerarium (GAS 198)	(190.0)	156.6	4-6(7)	12.0 (13.0)	10.9	Crust
			(5) 6–7	(10) 11.0–14.0 (15) × (8)	$12,2 \times$	
A. camerarium (GAS S/N)	110.0-140.0	125.5	(8)	9.0–12.0 (13)	10,3	Cortex

\* The feature was not observed.

Figure I. Macroscopic view of *Amauroderma calcitum* and related species. a-f *A. calcitum*: a- general view (DHCR 105, holotype); b- detail of hymenophore (holotype); c- general view (DHCR 110); d- detail in contexto and tubes (DHCR 105, holotype); e- detail in pilear surface (DHCR 110); f- section of basidiome (LPS 069). g- general view of *A. torrendi* = *A. calcigenum* (holotype, US 307270). h-k *A. partitum*:h-general view (type, K180681); i-detail of context and tubes (CS 296); j- detail of hymenophore (CS 022); k- general view (CS 022). Scale bar 1 cm.



Figure II. Spores of *Amauroderma calcitum* and related species. a-c *A. calcitum*: a-Scanning electron micrograph of basidiospores (GAS 074); b, c-basidiospores (DHCR 105, holotype). d, e- basidiospores of *A. calcigenum* (Robledo 1567). f, g- basidiospores of *A. partitum* (CS 296). Scale bar: a 2  $\mu$ m; b, d, f 10  $\mu$ m; c 5.7  $\mu$ m; e 6.9  $\mu$ m; g 4.6  $\mu$ m.



Figure III. Macroscopic view of *Amauroderma camerarium*. a, g type of *Polyporus omphalodes* (NY 730851): a- general view; g- pore surface in detail. b, i SP 61541: b- general view; i- pore surface in detail. c, h GAS 131:c- general view; h- pore surface in detail. d general view of GAS 172. e, f GAS 475: e-section of basidiome; f- pilear surface in detail. J- pore surface in detail of GAS s/n. Scale bar a-f 1 cm; g-j 1 mm.



Figure IV. Microscopic structures of *Amauroderma camerarium*. a, f, g type of *Polyporus omphalodes* (NY 730851): a- pilear surface as a crust; f, g-basidiospores. b, h, i SP 61541: b- pilear surface as a cortex; h, i- basidiospores. c, d, j, k GAS 131: c- pilear surface as an indeterminate derm; detail in clavate structures from pilear surface; j, k- basidiospores. f, l, m GAS s/n: f- pilear surface as a cortex; l, m- basidiospores. Scale bar:a-c, f 25 µm; d, f, h, j, 110 µm;



Figure V. Macrocopic view of basidiomata and spores of *Amauroderma* species from Cerrado. a, b, h *Amauroderma aurantiacum*: a- section of basidiome (LPS 025); b- pilear surface in detail (LPS 025); h- basidiospores (LPS 080). c, i, j *A. brasiliense* (DHCR 128). c – general view; i, j- basidiospores. d, e, k, 1 *A. praetervisum*. d- pilear surface in detail (LPS 066); e- section of basidiome (LPS 066); k, l- basidiospores (LPS 067). f, m, n *A. schomburgkii*. f- general view (GAS 393); m, n- basidiospores (GAS 393). g, o, p *A. spruce* (DHCR 114). g- general view; o, p- basiospores. Scale bar: a-g 1 cm; h, k, o 10 µm; i 8.7 µm; j 7.2 µm; 1 5.9 µm; m 10; n 3.1; p 2.9 µm.



CAPÍTULO II - AMAURODERMA OPACAESPITOSA SP NOV. (GANODERMATACEAE, AGARICOMYCOTINA) AND NOTES ON OTHER AMAURODERMA SPECIES RECORDED FOR ARGENTINA *Amauroderma opacaespitosa* sp nov. (Ganodermataceae, Agaricomycotina) and notes on other *Amauroderma* species recorded for Argentina.

#### Abstract

Amauroderma opacaespitosa is described and illustrated as a new from specimens collected in the Parque Nacional El Rey and Parque Nacional Calilegua in Yungas of Argentinean Northwest. This species is characterized by a caespitose habit, pale brown context without bands and broadly ellipsoid basidiospores. Besides that, Amauroderma sprucei are presented as new record for Argentina, and A. calcigenum and Amaurodema pseudoboletum for Yungas of Argentinean Northwest. A key to Argentinean species of Amauroderma is provided.

#### Key words

Taxonomy, Polyporales, Argentinean Yungas

## 1. Introduction

Amauroderma Murril presents a predominantly tropical distribution, comprising around 30 species, while 21 occur in neotropical region (Furtado 1981; Ryvarden 2004; Campacci and Gugliotta 2009; Gomes-Silva and Gibertoni 2012). The genus is characterized by polypores, usually stiptate and dull, in various shades of brown, with basidiopores globose to oblong, without a truncate apex, double walled with the inner layer ornamented (rarely smooth), associated with dead wood or roots of living or dead trees (Furtado 1981; Ryvarden 2004).

Since the taxonomical revision of the genus by Furtado (1981), which presented many proposals of synonymization, new combination and of new taxa, only two new combinations [Amauroderma brasiliense (Singer) Ryvarden and Amauroderma dubiopansum (Lloyd) Ryvarden], the synonymization of A. macrosporum Furtado in A. aurantiacum (Torrend) Gibertoni and Bernicchia and three new species, A. coltricioides Henkel, Aime et Ryvarden, A. deviatum Ryvarden and A. elegantissimum Ryvarden and Iturriaga, were proposed for the Neotropics (Ryvarden 2004; Gibertoni et al. 2008; Aime et al. 2003). In Argentina, Spegazzine (1926) recorded A. psedoboletum (Speg.) J.S. Furtado and Ibañez (1995) Amauroderma boleticeum (Pat. & Gaill.) Torr. and Amauroderma schomburgkii (Mont. & Berk.) Torrend. Wright (2005), in turn, suggested with records of Amauroderma aff. camerarium (Berk.) J.S. Furtado, Amauroderma aff. exile (Berk.) Torrend and A. aff. schomburgkii, that the Argentinean mycota should be more investigate in order to revel new species of Amauroderma.

During the taxonomical revision of mycota from Argentinean Northwest Yungas, some caespitose specimens of *Amauroderma*, with brown context without bands and broadly ellipsoid basidiospores seemed quite particular. After a detailed and comparative study of such specimens and reference material, including the literature, we realize that these specimens represent an undescribed species of *Amauroderma*. In this context, we present hereafter the new taxon, some new records of *Amauroderma* species for Argentina and for Argentinean Northwest Yungas. Description, taxonomic notes and distribution comments about of the species are presented as well.

## 2. Materials and methods
Field expeditions were carried in the phytogeographic province of Yungas, in Argentinean northwest, into the parks: Parque Nacional El Rey, Parque Nacional Calilegua and Parque Nacional Baritú. Specimens studied are preserved at CORD. Reference material from BPI, SP and URM herbaria were used to morphological comparison. Herbarium acronyms followed Thiers B. [continuously updated].

Macro and microscopically analyzes of basidiomata follows Teixeira (1995) and Ryvarden (2004). Microscopic examinations and measurements were done in an Olympus BH2 phase contrast microscope, using Melzer's reagent. In presenting the sizes of the microscopic elements of the studied specimens, 5% of the measurements were excluded from each end and are given in parentheses;  $\bar{\mathbf{X}} =$ arithmetic mean of basidiospores;  $\mathbf{Q} =$  the ratio of length/width of basidiospores;  $\bar{\mathbf{X}}_{\mathbf{Q}} =$  arithmetic mean of the ratio. Forty basidiospores were measured.

For ultrastructural observations, both basidiospores with and without exospore were observed. In the first case, fragments of tubes were place on stubs then metalized with golden and observed. Removing the outer layer, to observe the spore ornamentation in detail, fragments of dissepiment were placed on chromic acid (H<sub>2</sub>CrO<sub>4</sub>) crystal, covered by enough water drops to dissolve the crystals, and stored around 20 minutes. Then, this solution and dissepiment fragments were filtered (0,45 µm filter) by vacuum, adding with water to remove acid. The filter was dried at room temperature and finally scraped with a blade in a stub with a drop of 70% alcohol, metalized with golden and observed at SEM. The analyses were performed at in Scanning Electronic Microscope (SEM) Zeiss LEO 1450VP of the Laboratorio de Microscopia Electronica y Microanalisis (LABMEM) of the Universidad Nacional de San Luis, Argentina.

#### 3. Taxonomy

Amauroderma opacaespitosa Robledo, Rajchenberg and Costa-Rezende sp. nov.

Fig. 1

Basidiomata annual, sitipitate, caespitose. Pilear surface brown and dull. Context brown in pileus and stipe. Pores angular, 4/mm. Hyphal system dimitic. Generative clamped and skeleto-binding hyphae, straw to brownish, thick-walled, IKI-. Basidiospores ellipsoid,  $10 - 11 \text{ x} 8 - 9.5 \text{ }\mu\text{m}$ .

Holotype: Argentina, Salta, Anta, Parque Nacional El Rey, 10-Marzo-2005, *Robledo 499* (CORD, isotype FLOR).

Etymology: "opaca" dull and "caespitosa" caespitose, referring to the particular caespitose habit and the dull pale brown color of basidiomata.

Basidiomata annual, light-weight, caespitose; pileus circular, centrally depressed, convex to strongly incurved when dried composed by fusion of reniform or flabelliform portions, with independent stipes, sometimes not completely fused or spathulate, then away from the main group of pileus, or fused from the stipe with also fused pileus, up to 6 cm in diameter and 0,5 cm thick. Pilear surface glabrous, dull, pale gravish brown, concentrically zonated with paler shades, radially and finely fibrillose under the lens, concentrically undulate to rugose, some with remarkable radial wrinkles and warts in the depressed central area. Margin acute, incurved when dried, concolor to pilear surface. Pore surface whitish when fresh becoming dark ochraceous brown when dried. Pores slightly decurrent but clearly delimited on the stipe, angular, 4/mm, radially elongated in the decurrent portion. Disseptiments entire. Context dull, brown, homogeneus, up to 0,4 cm thick, with a distinct darker zone in the upper part of the base of pileus. Tubes concolor to the context, up to 0,3 cm deep. Stipe solid, cilindric, radially rugose and irregularly flattened, straight to geniculated, up to 6 cm above the ground, with up to 5 cm buried, up to 0,9 cm in diameter near the pores, getting thinner towards the base, sometimes branches or stipes without pileus are observed in caespitose portions; stipe surface slightly darker than pilear surface, glabrous to slightly velutinose under the lens, in section with context concolor to pilear surface, becoming paler in the center, straw colored.

*Hyphal system* dimitic. *Context* composed by clamped generative hyphae, hyaline, thin to slightly thick-walled,  $2 - 5 \mu m$  in diameter; skeleto-binding hyphae, terminal or intercalary, pale brown to yellowish, thick-walled, with a broad lumen, formed by a long main stalk (up to 850  $\mu m$ ) and lateral branches, with various length and degrees of ramification, most of that perpendicular to the main stalk, often with two branches in one or both apex,  $5 - 7 \mu m$  in diameter in

main stalk, 2- 3 µm in terminal branches; arboriform skeletal hyphae rare, pale brown to yellowish, thick walled, straight to tortuous and geniculate, often with two apical branches that gives raise to few thinner terminal branches, up to 6 µm in diameter in the main stalk. Trama of tubes composed by clamped generative hyphae, hyaline, thin to slightly thick-walled,  $2-5 \mu m$  in diameter; skeleto-binding hyphae, terminal or intercalary, pale brown to yellowish, almost solid, formed by a main stalk, shorter than contextual  $(150 - 500 \mu m \log)$  and lateral branches, with various length and degrees of ramification (in general with shorter and less ramified branches than context), most of that perpendicular to the main stalk, often with two branches in one or both apex,  $5 - 7 \mu m$  in diameter in main stalk, 2-4 µm in terminal branches. Pilear surface near the depressed portion composed by clamped generative hyphae, slightly thick walled, with a broad lumen and an anticlinal disposition; towards the margin without any difference form context, non to few agglutinated, IKI-.

Cystidia absent. Cystidioles fusoid, 25-30 x 5-10 µm.

Basidia clavate, 30-40 x 15-17 µm, with four sterigmata.

Basidiospores mostly broadly ellipsoid,  $10 - 11.5 \ge 8 - 9.5$  (-10)  $\mu$ m,  $\bar{x} = 10.8 \ge 8.9 \mu$ m, Q = 1.10 - 1.28 (1.38),  $\bar{x}_Q = 1.21$ , with delicate circular to slightly elongated endosporic projections, pale yellowish brown to reddish brown, IKI-.

**Ecology and distribution** - Species growing on the ground, collected only in Argentinean northwest. Commonly collected in low and dry open lands, in Parque Nacional El Rey, also collected in Parque Nacional Calilegua, in moist forests. Most of the specimens were collected in march (only one was collected in may), it might represent that the species presents a restrict phenology or ephemeral basidomata.

**Specimens examined** – Argentina, Jujuy, Dpto. Ledesma, Parque Nacional Calilegua, Sendero La Lagunita, 23°45'18"S, 64°51'13"O, alt. 717 msnm, 06-Mar-2005, 06 Mar 2005, Robledo 484 (CORD), in dead branch on soil; *Ibidem*, Salta, Dpto. Anta, Parque Nacional El Rey, entrada, 25°00'34"S, 64°35'55"O, alt. 868 msnm, 10 Mar 2005, Robledo 499 (CORD holotype), on the ground; *Ibidem*, Los Lobitos, 24°41'40,0"S, 64°36'43,7"O, alt. 876 msnm, 25 Mar 2007, Robledo 1089 (CORD), on the ground.

**Other specimens examined** – Amauroderma praetervisum. Brazil, sine datum, Weddel (BPI 237178, isotype); A. camerarium. Ibidem, Amazonas, Panuré, 00.03.1853, Spruce 171 (BPI 327148, syntype); Ibidem, Mato Grosso, Aripuanã, Cachoeira Dois Irmãos, 06.07.1962, M. Mee (SP 61451): A. omphalodes. Ibidem, sine datum, Spruce 32 (NY 730851, type); A. elegantissimum. Ibidem, Rondonia, Porto Velho, Estação Ecológica de Cuniã, 11.02.2011, Gomes-Silva (URM 82788).

**Taxonomic remarks** - *Amauroderma opacaespitosa* is a remarkable species due to a combination of caespitose basidiomata, coriaceous and dull pileus, pale brown context without black bands and broadly ellipsoid spores with delicate circular to slightly elongated endosporic projections. The caespitose habit is an unusual feature in the genus, non-observed by Furtado (1981), who pointed out that pilear association has not a meaningful value in species delimitation of *Amauroderma*. Ryvarden and Johansen (1980) mentioned that African specimens of *Amauroderma expallens* (Bres.) Furtado are characteristic by its several fused pileus in the same stipe. This feature was not observed by Bresadola (1925), as not reported in the original description, and neither by Furtado (1981). *Amauroderma expallens* presents smaller globose to subglobose spores,  $7 - 9 \ge 6 - 9 \ \mu m$  and  $8,5 - 10,5 \ge 6 - 8 \ \mu m$ , as reported by Furtado (1981) and Ryvarden and Johansen (1980), respectively.

Among neotropical species, Amauroderma camerarium (Berk.) J. Furtado, Amauroderma elegantissimum Ryvarden & Iturr. and Amauroderma praetervisum (Pat.) Torrend share a pale brown context (at least in old specimens), small pores and broadly ellipsoid (up to ellipsoid in A. elegantissimum) basidiospores with A. opacaespitosa. However, the first species presents two distinct bands in the context, besides larger spores, (10) 11 - 14 (15) x (8) 9 - 12 (13) µm, with very conspicuous endosporic ornamentation. Amauroderma elegantissimum presents a very thin pileus and larger spores, 12 - 15 (16) x 8 - 10 µm as well, and A. praetervisum also presents black bands in the context, a crust in pilear surface and wider spores (10 - 13 µm).

#### Notes on other Amauroderma species recorded in Argentina

Amauroderma calcigenum (Berk.) Torrend

#### Complete description in Ryvarden 2004

**Ecology and distribution -** Neotropical species founded growing on the ground, (Furtado 1981; Ryvarden 2004). Previously recorded to Argentinian northeast (Popoff 2000). It is the first record to Yungas of Argentinian northwest, where the specie has been frequently collected in Parque Nacional Calilegua.

**Specimens examined** – Argentina, Jujuy, Dpto. Ledesma, Parque Nacional Calilegua, Sendero Momota, 23°48'48,1"S, 64°51'1,5"O, alt. 600 msnm, 07 Mar 2005, Robledo 394 (CORD); *Ibidem*, Sendero Tataupá, 23°44'12"S, 64°50'60"O, alt. 800 msnm, Robledo 1141 (CORD), on the ground; *Ibidem*, Mesada de las colmenas, Sendero La Cascada, 23°42'1,5"S, 64°51'56,8"O, alt 1082 msnm, 22 May 2007, Robledo 1567 (CORD), on the ground; *Ibidem*, Sendero Pedemontano, 02 Apr 2008, Robledo 1875 (CORD), on the ground.

**Other specimens examined** – *A. calcigenum.* Brazil, *sine datum*, Gardner (BPI237146, isotype); Brazil, Goiás, Crixás, *sine datum*, A. Brookes (BPI 302396, type of *A. brockesii*); Brazil, Bahia, *sine datum*, C. Torrend (BPI 307270, type of *A. torrendii*).

**Taxonomic remarks** – Amauroderma calcigenum is easily distinguished due to its reddish thick pileus, large angular pores (1-2/mm) and large ellipsoid spores (14-) 15-16 x (10-) 11-12  $\mu$ m. The Yungas herbarium material LPS 25771 (October 1917, Spegazzini), previously recorded as Amauroderma macrosporum from by Robledo and Rajchenberg (2007) is in fact an specimen of *A. calcigenum*. Amauroderma aurantiacum is macroscopically similar to *A. calcigenum* due to its thick and sometimes reddish pileus, large pores (1-2/mm) and large basidiospores. However, the spores of *A. aurantiacum* are globose to subglobose with a subreticulate ornamentation pattern. Amauroderma partitum (Berk.) Wakef is also morphologically related, sharing a redish pilear surface and big ellipsoid spores, differing due to thinner and pliable basidiomata and smaller spores 10–13 x (7) 8–10 (10,5)  $\mu$ m.

Amaurodema pseudoboletum (Speg.) J.S. Furtado

Complete description in Furtado (1981)

**Ecology and distribution** –Neotropical species founded growing on the ground (Furtado 1981; Ryvarden 2004). It was already recorded for Argentina by Spegazzini (1926), but not for Argentinian northwest Yungas.

**Specimens examined** – Argentina, Salta, Dpto. Sta. Victoria Oeste, Parque Nacional Baritú, Lipeo 22°26'21,6"S, 64°44'7,8"O, alt. 1222 msnm, 06 May 2007, Robledo 1253 (CORD), on the ground; *Ibidem*, trail to Campo Grande, 22°26'10,8"S, 64°43'40,2"O, Alt 1190 msnm, 09 May 2007, Robledo 1441 (CORD), on the ground.

**Other specimens examined** – *A. praetervisum.* Brazil, sine datum, Weddel (BPI 237178, isotype); *A. camerarium. Ibidem*, Amazonas, Panuré, 00.03.1853, Spruce 171 (BPI 327148, syntype); *Ibidem*, Mato Grosso, Aripuanã, Cachoeira Dois Irmãos, 06.07.1962, M. Mee (SP 61451): *A. omphalodes. Ibidem, sine datum*, Spruce 32 (NY 730851, type); *A. pseudoboletum.* Paraguay, Guarapi, 04.1879, Spegazzini (BPI 237180, isotype); Brazil, São Paulo, Cantareira, Horto Florestal, 28.03.1962, J.S. Furtado (SP 61135).

**Taxonomic remarks** – Amauroderma pseudoboletum is characterized by its brown context with two dark bands, dextrinoid skeletal hyphae in the tubes and large globose to subglobose spores, 12 -14 (-15) x (10-) 11 -13 µm, with conspicuous circular endosporic projections, pale yellowish brown. Amauroderma camerarium (Berk.) J. Furtado and A. praetervisum (Pat.) Torrend present a pale brown to brown context with dark bands as well, variable dextrinoid skeletal hyphae and similar size of basidiospores. On the other hand, A. *camerarium* presents smaller pores ( $80 - 230 \mu m$  diam.), 5 - 7pores/mm, thicker dissepments  $(50 - 230 \mu m)$  and usually paler context than A. pseudoboletum. Amauroderma praetervisum presents broadly ellipsoid spores and not so conspicuous ornamentation. Despite of pseudoparenquimatous structures in the pilear surface had not been reported by Furtado (1981) and Ryvarden (2004), and not observed in the type collection of A. pseudoboletum, as well pores diameter and dissepiments thickness could not be measured (the specimen seems to had been pressed), the combination of a brown context, dextrinoid skeletal hyphae and large globose to subglobose basidiospores with conspicuous ornamentation is not observed in other neotropical Amauroderma species (except A. camerarium, discussed above), which led us to identify the specimens as A. pseudoboletum.

Amauroderma sprucei (Pat.) Torrend

Complete description in Decock and Herrera Figueroa (2006)

**Ecology and distribution** –Neotropical species founded growing on the ground (Furtado 1981; Ryvarden 2004; Decock and Herrera Figueroa 2006) and is recorded here the first time for Argentina.

**Specimens examined** – Argentina, Jujuy, Depto Ledesma, Parque Nacional Calilegua, Abra de Cañas, 23°40'38,2"S, 64°53'46,3"O, alt. 1730 msnm, 21 May 2007, Robledo 1507 (CORD), dead stump.

**Other specimens examined** - Brazil, Panure, *sine datum*, Spruce 44 (BPI 237203, isotype)

**Taxonomic remarks** – The whitish context, small pores [(4-) 5 (-6) /mm], pilear surface composed by a layer of agglutinate generative hyphae, without organization, forming a trichoderm, dextrinoid skeletal hyphae and subglobose and conspicuous columnar ornamented spores [9 – 10 (-10,5) x 8 – 9 (-9.5) µm] are characteristic of this species. In this work we follow the morphological concept of Decock and Herrera Figueroa (2006). Considering the pale context, coriaceous to subwood consistence of pileus and size of pores, *A. camerarium* and *A. praetervisum* could be morphologically related. Both species present dark bands in context and large spores, (10) 11 – 14 (15) x (8) 9 – 12 (13) µm and (11.5) 12 – 13.5 (14) x 10 -12 (13) µm, respectively. *Amauroderma schomburgkii* also presents similar pilear consistence, small pores and basidiospores with similar shape and size, differing mainly due its golden brown context and a cortex in pilear surface.

#### Key to Amauroderma species recorded for Argentina

1 Hymenophore with up to 4 pores/mm	2
1' Hymenophore with more than 4 pores/mm	5

2 Basidiospores up to 12 μm in longest dimension
3 Basidiomata single, basidiospores globose to subglobose
A. boleticeum 3' Basidiomata caespitose, basidiospores broadly ellipsoid 
4 Skeletal hyphae dextrinoid, basidiospores predominantly globose to subglobose
A. <i>pseudobletum</i> 4' Skeletal hyphae non-dextrinoid, basidiospores distinctly ellipsoid <i>A.</i> <i>calcigenum</i>
5 Context golden brown, pilear surface cortex, skeletal hyphae non- dextrinoid

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Figure 1. Basidiomata of *Amauroderma opacaespitosa*. A, C, D, E, F Robledo 1089. B Robledo 484. A, B- general view. C- pilear surface. Dhymenophore. E- detail in context. F- detail in hymenophore. Scale bar: A-E 1 cm; F- 1 mm.



Figure 2. Basidiospores of *Amauroderma opacaespitosa*. A, B- optical microscopy (KOH and Cotton blue, respectively). C-F SEM micrographs. C- general view showing exospore without holes. D-general view, with total exospore remotion, showing solid collumns and smaller secondary ornamentation of the endospore. E- side view of broken basidiospore, showing the collumns reaching the exospore and smaller secondary projections. F- Detail in solid collumns and smaller secondary ornamentation of the endospore. Scale bar: A, B- 10  $\mu$ m; C- 3  $\mu$ m; D-  $\mu$ m; 2 E,F- 1  $\mu$ m.



CAPÍTULO III - FORAMINISPORUS GEN. NOV. AND A PHYLOGENETIC APPROACH OF AMAURODERMA AND RELATED GENERA

# *Foraminisporus gen. nov.* and a phylogenetic approach of *Amauroderma* and related genera

**Abstract:** Ganodermataceae is a remarkable group of polypore fungi, being mainly characterized by the double-walled spores, with ornamented inner layer. Ultra-structural and phylogenetic studies raised up a new character for Ganodermataceae systematics, which are the hollow columns of the endospore. This feature is considered a new taxa synapomorphy and *Foraminisporus* gen. nov., is proposed to accommodate *Amauroderma sprucei*. The first phylogenetic approach of *Amauroderma* is presented, as well comments of relationship with related taxa.

*Key words:* Ganodermataceae, Polyporales, Ultrastructure, Systematics.

#### INTRODUCTION

Ganodermataceae is mainly characterized by pileate basidiomata, sessil to stipitate, hyphal system dimitic, with arboriform and skeletobinding hyphae and basidiospores double walled, with the inner ornamented, an unique feature of the taxa. The family presents a cosmopolitan distribution, with around 220 species (Moncalvo & Ryvarden 1997). The Ganodermataceae species cause a white rot in wood or are associated with roots of live or dead trees (Ryvarden 2004).

Family taxonomy was almost exclusively based on morphological characteristics, such as appearance of pilear surface, disposition of the hyphae in pilear surface and basidiospores features (shape and ornamentation pattern), including some ultrastructural approaches on basidiospores (Torrend 1920; Furtado 1962; Furtado 1965, 1981; Steyart 1972; Steyart 1977; Ryvarden & Johansen 1980; Corner 1983; Gottlieb & Wright 1999 a,b; Ryvarden 2004; Torres-Torres & Guzmán-Dávalos, 2012). In this way many discussions have been raised about generic and infrageneric level in the family.

The most accepted genera are Amauroderma Murrill, Ganoderma P. Karst, Haddowia Steyart, Humphreya Steyart and Tomophagus Murrill (Teixeira 1994; Moncalvo 1996; Moncalvo & Ryvarden 1997; Ryvarden 2004; Kirk et al. 2008, Le et al. 2012). Ganoderma is characterized by ellipsoid to ovoid basidiospores, with truncate appex and columnar ornamentation. Humphreya presents basidiospores with same shape, but with different ornamentation, which is composed mainly by logitudinal rigdes. Tomophagus also presents spores with truncate apex, but with a pale and soft context. Amauroderma and Haddowia present basidiospores without truncate appex, being different mainly due to ornamentation pattern, which is columnar to semireticulate ornamentation in Amauroderma and longitudinal ridges ornamentation in Haddowia (Furtado 1981; Steyaert 1972; Ryvarden 2004; Le et al. 2012). In addition, other genera with ganodermatoid spores were proposed, however widely recognized as synonyms of Ganoderma or Amauroderma, such as Elfvingia P. Karst and Magoderma Stevaert (Ryvarden 1991; Moncalvo & Ryvarden 1997).

The phylogenetic relationship of Ganodermataceae, at genera level, remains unclear. There are few phylogenetic works and most of them are takes into account many specimens of *Ganoderma* and few or none of the other genera. In a general view, the genera and the family have been pointed out as monophyletic, paraphyletic and polyphyletic in the works already carried out (Moncalvo et al. 1995; Moncalvo 1996; Hong & Jung 2004; Glen et al. 2009; Justo & Hibbett 2011; Cao et al. 2012; Binder et al. 2013).

Examining the ultrastructure of some neotropical specimens of *Amauroderma*, previously determined as *A. sprucei* (Pat.) Torrend, a quite particular endosporic ornamentation was observed and some of the columns-like endosporic projections are perforated, and that holes persisting until the exospore. Besides that, phylogenetic analyses showed that *A. sprucei* form a separate lineage from *Ganoderma* and *Amauroderma*. In the framework to establish a natural classification of Ganodermataceae, based on both morphological and molecular analysis, a new genus is proposed, up to now, to accommodate *A. sprucei*.

#### MATERIAL AND METHODS

Specimens and morphological studies.— The studied specimens are deposited in FLOR and CORD herbaria. Herbarium acronyms follow Thiers B. (continuously updated). Microscopic examinations and measurements were done using Melzer's reagent, Cotton blue and/or 3-5% KOH as mounting media. Spores-wall designations follow the concept of Furtado (1962). In presenting the sizes of the microscopic elements of the new species, 5% of the measurements were excluded from each end and are given in parentheses; forty basidiospores were measured.

For ultrastructural observations, both basidiospores with and without exospore were observed. In the first case, fragments of tubes were place on stubs then metalized with golden and observed. Removing the outer layer, to observe the spore ornamentation in detail, fragments of dissepiment were placed on chromic acid (H<sub>2</sub>CrO<sub>4</sub>) crystal, covered by enough water drops to dissolve the crystals, and stored around 20 minutes. Then, this solution and dissepiment fragments were filtered (0,45 µm filter) by vacuum, adding with water to remove acid. The filter was dried at room temperature and finally scraped with a blade in a stub with a drop of 70% alcohol, metalized with golden and observed at SEM. The analyses were performed at in Scanning Electronic Microscope (SEM) Zeiss LEO 1450VP of the Laboratorio de Microscopia Electronica y Microanalisis (LABMEM) of the Universidad Nacional deSan Luis, Argentina and JEOL JSM-6390LV, provided by the Laboratório Central de Microscopia Eletrônica of the Universidade Federal de Santa Catarina (Brazil).

DNA extraction and sequencing.— For extraction of total genomic DNA from dried basidiomata protocol of Doyle & Doyle (1987) adapted by Góes-Neto et al. (2005) was used . Primers pairs ITS6-R and ITS8-F (Dentinger et al. 2010) and LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers. htm) were used to polymerase chain reaction . Sequencing was performed with *BigDye Terminator v.3.1 Cycle Sequencing Kit* following manufacturer procedures. For sequencing ITS region were used the primers cited, while for LSU region, the primers LR0R and LR5 were used (http://www.biology.duke.edu/fungi/mycolab/primers. htm). The sequencing was performed at Centro de Pesquisas René Rachou Fiocruz (Brazil). The newly generated sequences and additional sequences downloaded from GenBank are listed in the Table I.

Phylogenetic analyses.— Sequences were manually edited using Genious software (Biomatters) and alignment was made using MAFFT (Katosh et al. 2009), following the G - INS - i criteria; ambiguous regions were identified with GUIDANCE (Pen et al. 2010), using 100 bootstrap replications, and positions with confidence index less than 60% were removed from the final alignment. Three datasets, ITS and LSU separately and a combined ITS+LSU are generated and used for phylogenetic analysis. Finally, datasets were manually inspected using MEGA v.6 (Tamura et al. 2013). The best fit model of nucleotide evolution to each dataset was selected with BIC (Bayesian Information Criterion) using Jmodeltest software (Guindon & Gascuel 2003; Darriba et al. 2012). Maximum Likelihood (ML) and Bayesian Inference (BI) were applied to the three datasets. Bayesian Inference were performed using BEAST v.2 software (Bouckaert et al. 2014), with twice random run, with 10000000 replications for ITS and LSU and 20000000 for combined dataset), and sampling the best tree every 1000 generations. Stability of each race was tested following ESS (Effective Sample Size) criteria using Tracer v.1.4 software (Rambaut & Drummond 2007). Consensus tree was generated with a 10% burnin in TreeAnotator v.2.1.2 (Bouckaert et al. 2014). Maximum Likelihood analyses were realized using RaxML software, available in the Cipres platform, with five categories gamma and 500 pseudoreplications (bootstrap).

RESULTS

*Molecular Phylogeny*—ITS dataset included sequences from 47 fungal specimens (22 taxa), representing a length of 544 characters, which 352 are conserved. The LSU dataset included sequences from 31 fungal specimens (16 taxa), representing a length of 747 characters, which 674 are conserved. The combined (ITS+LSU) dataset included sequences from 23 fungal specimens (14 taxa), representing a length of 1289 characters, which 1069 are conserved. The best models for ITS, LSU and LSU+ITS datasets were K80+I+G, TrNef+I and K80+I+G/TrNef+I. respectively.

ML analyses of ITS is not presented and discussed because did not present statistic support value in most of clades. On the other hand, Bayesian analyses of ITS reveled both *Amauroderma* and *Ganoderma* as not monophyletic groups. Four main clades (fig. 1, here presented from top to bottom) are demonstrate: 1. *Amauroderma* s.s. clade (0,98 PP) composed by the core of the genus; 2. *Ganoderma* clade (0,3 PP); 3. *Foraminisporus* clade (0,97 PP) composed by three specimens of *A. sprucei*; and 4. *Tomophagus* clade (0,66 PP value) with two *Tomophagus* species (including the type species *T. colossus*), *G.* aff. *guianensis* and a long branch with *A. brasiliense* (Singer) Ryvarden.

Amauroderma s.s. clade is divided in four smaller clades: one (0,69 PP) composed by A. auranticum (Torrend) Gibertoni & Bernicchia, A. schomburgkii (Mont. & Berk.) Torrend, A. aff. schomburgkii and A. aff. praetervisum (Pat.) Torrend; the second (0,95 PP) composed by A. camerarium (Berk.) J.S. Furtado, A. partitum (Berk.) Wakef and A. calcitum Costa-Rezende & Drechsler-Santos (unpublished, chapter 1); the third (0,59 PP), with one specimen of A. calcigenum (Berk.) Torrend; and the last one (0,8 PP) with A. intermedium (Bres. & Pat.) Torrend, A. pseudoboletum (Speg.) J.S. Furtado and Amauroderma sp.

Ganoderma clade is composed by Ganoderma lobatum [Schwein.] G.F. Atk and G. australe [Fr.] Pat., species with a dull pilear surface (0,99 PP); G. lucidum [Curtis] P. Karst., species with laccate pilear surface and Humphreya coffeata (Berk.) J.S. Furtado (0,66 PP); and A. rude (Berk.) Torrend (1,00 PP).

Bayesian analyses of LSU also presented four main clades, with *Amauroderma* as polyphyletic (fig. 2, here presented from top to bottom): 1. *Foraminisporus* clade. (0,36 PP) composed by two specimens of *A. sprucei*; 2. *Amauroderma s.s.* clade (0,96 PP) composed by *A.* aff. *partitum* and *A. camerarium* as a sister clade of *A. schomburgkii* specimens (1,00 PP) and by *A. aurantiacum* and *A.* 

*intermedium* (0,74 PP); 3. *Ganoderma* clade (1.00 PP) composed by *G*. cf. *australe* (dull species), laccate species (*G. boninense* Pat, *G. lucidum*, *G. microsporum* R.S. Hseu and *G. tsugae* Murrill), *G. australe* (among the laccate species), and a stipitate laccate specimen, that nested as sister of these cited above; 4. *Amauroderma brasiliense* clade, a long well supported (1,00) branch.

ML analyses (not shown) presents a similar topology, except by *A. aurantiacum* that nested as a sister clade (26% bootstrap value) of an grouping composed by *A. calcitum*, *A. camerarium* and *A. schomburgkii*; by *A. intermedium* and *A. brasiliense* that nested in a sister clade of *Ganoderma* (23% and 18% bootstrap value, respectively); by *A. sprucei* that falls in a polytomy; and some differences in the arrangement of species in *Ganoderma* clade with very low support.

Bayesian inference of ITS+LSU (fig. 3) also presents four main clades, similar those other analyses, where *Amauroderma* is recognized as non-monophyletic entity. *Amauroderma s.s.* clade (0,99 PP and 74% bootstrap value), sub-divided in three clades, one composed by *A.* aff. *partitum* (Berk.) Wakef and *A. camerarium* (Berk.) J.S. Furtado (1,00 PP and 100% bootstrap value) and a sister clade of *A. schomburgkii*; the second (0,99 PP and 75% bootstrap value), composed by *A. aurantiacum*, nested as a sister group of the clade referred above; and other (0,99 PP and 74% bootstrap value) composed by *A. intermedium*.

Ganoderma clade receive a high support both for BI and ML (1,00 PP and 99 % bootstrap value) and showed two well supported smaller clades, one composed by laccate species (*G. boninense, G. lucidum, G. microsporum* and *G. tsugae*) and other composed by three specimens with a dull pilear surface that fits in morphological sense of *G. australe. Foraminisporus* clade with one specimen of *A. sprucei* (0,84 PP) and finally, *A. brasiliense* clade (1,00 PP).

Differences between BI and ML (fig 4) analyses were in respect to the position of *A. brasiliense* and *A. sprucei*; in the BI, *A. sprucei* nested as a sister clade of *Ganoderma* and *Amauroderma* (0,84 PP) and *A. brasiliense* external to this hole group (1.00 PP); in the ML, these taxa nested in inverse position, *i.e.*, *A. brasiliense* appeared as sister clade of *Ganoderma* and *Amauroderma* (47% bootstrap value) and *A. sprucei* external to this hole group (100% bootstrap value); and MABS 303 (*Ganoderma cf australe*) wich nested as a sister clade of the clades referred above. In all topologies, Ganodermataceae appeared as monophyletic and *Amauroderma* and *Ganoderma* dichotomies always received low support values (< 0.50 PP and 50% bootstrap).

#### TAXONOMY

*Foraminisporus* gen. nov. ad. int. Robledo, Costa-Rezende & Drechsler-Santos Fig. 5 A-F

*Basidiomata* annual, stipe pleuropodal to pseudomesopodal, pileus circular to spathulate. *Pilear surface* glabrous, greyish brown to dark brown, concentrically zonate with thin blackish bands, radially rugose. *Context* white, homogenous, in section with a shiny black cuticle. *Tubes* slightly darker than context. *Pore surface* whitish to vivid orange. *Pores* regular, circular to angular. *Dissipments* thick, entire. *Stipe* cylindrical, pale to dark brown, finely tomentose, solid to hollow, context homogeneous whitish, in section with a shiny black cuticle. *Hyphal system* dimitic, generative hyphae clamped, arboriform and skeleto-binding hyphae almost hyaline, dextrinoid. *Cystidia* and *cystidioles* absent. *Basidia* clavate, with four sterigmata. *Basidiospores* subglobose, hyaline to pale brown, with conspicuous ornamentation as endosporic projections column-like, some of them, with a hole, that persists until the exospore.

*Etymology.*— *Foramen*- hole, *sporus*-spore, referring to the spores with hollow projections in the endospore.

*Typus.— Foraminisporus sprucei* (Pat.) Costa-Rezende, Drechsler-Santos & Robledo

*Ecology and distribution.*— Specimens usually associated with roots of living trees. Brazil, Venezuela, French Guyana, Costa Rica, Belize and Cuba (Decock & Herrera-Figueroa 2006).

*Remarks.— Foraminisporus* is characterized by having a stipitate basidiomata, dull pilear surface, whitish context, a dimitic hyphal system, skeleto-binding hyphae with lateral and apical branches and arboriform skeletal hyphae, both dextrinoid, and globose to subglobose, hyaline to pale brown spores, with conspicuous endosporic projetions. Under SEM, is possible to observe that some of columnar endosporic

projections are hollow, with this holes persisting until the exospore wall. This feature is unique for Ganodermataceae, thus considered a

synapomorphy for the genus. At family level, the genus clearly fits into Ganodermataceae circumscription, due to hyphal system with clamped generative and arboriform skeletal hyphae, as well, double walled basidiospores, with the inner layer ornamented. Both macro- and microscopic features of Foraminisporus are shared with the genus Amauroderma Murrill, i.e., stipitate and annual basidiomata, a dimitic hyphal system and basidiospores non-truncate (Furtado 1962, 1981; Ryvarden & Johansen 1980; Corner 1983; Ryvarden, 2004). However, an ultra-structural examination of some species of the genus [including the type specie of Amauroderma (Amauroderma schomburgkii)] led us to conclude that the perforated column is absent in the genus (Figs. 6-8). Since only F. sprucei is known with this feature, its whitish context and the vivid orange pore surface seem to be remarkable features of the genus in its actual circumscription, nevertheless the whitish context is also presents in some Amauroderma species (at least in a part of development) and the vivid orange spores is not observed in many specimens of F. sprucei.

Ganoderma Karst., considering both laccate and dull species, also presents species with pale context and double-walled spores with endosporic ornamentation (Ryvarden & Johansen 1981; Corner 1983; Ryvarden 2004; Torres-Torres & Guzmán-Dávalos 2012), however, the absence of the hollow columns (Gottlieb & Wright 1999 a,b) and the truncate apex of basidiospores are characteristics that clearly distinguish this genus from Foraminisporus. Pegler & Young (1973) and Steyart (1977), using SEM micrographs, also noted the presence of holes in the exospore of some species of Ganoderma, nevertheless in these cases, the holes are formed among the columns, differing from Foraminisporus. Haddowia and Humphreya also present species with pale context and ornamented endospore, however the ornamentation is formed by mainly longitudinal ridges for both. Additionally, Humphreya also presents truncate spores. Tomophagus differs from Foraminisporus by its laccate and soft pileus and truncate spores (Murril 1905; Stevart 1972; Ryvarden 2004; Le et al. 2011).

The ontogenetic formation of endosporic ornamentation in Ganodermataceae is unexplored up to now, but its should be investigate in order to contribute to the taxa delimitation in Ganodermataceae. It was corroborate in other polypore groups already observed, such as in *Perenniporia s.l.* (Decock & Ryvarden 2003). In this context, we

believe that formation of hollow columns is a good a character to delimit *Foraminisporus* as a distinct genus.

*Foraminisporus sprucei* comb. nov. Costa-Rezende, Drechsler-Santos & Robledo

Basionym.— Ganoderma sprucei Pat.

Synonyms.— ≡Ganoderma sprucei Pat., Bull. Soc. Mycol. Fr. 10:75. 1894.

*≡ Porothelium rugosum* Berk., J. Bot. & Kew Misc. 8:237, 1856.

= *Polyporus dubiopansus* Lloyd, Lloyd Myco. Writ. 3:125, 1921.

*≡Amauroderma dubiopansum* (Lloyd) Ryvarden, Neotropical Polypores, Synop. Fung. 19:52, 2004.

Description.— Decock and Herrera Figueroa (2006) as Amauroderma sprucei.

*Distribution.*— Brazil, Venezuela, French Guyana, Costa Rica, Belize and Cuba (Decock & Herrera-Figueroa 2006, Campacci & Gugliotta 2009, Drechsler-Santos et al. 2013, Gugliotta et al. 2014).

Specimens examined.— BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 07 January 2013, D.H. Costa-Rezende 113 (FLOR); 07 January 2013, D.H. Costa-Rezende 114 (FLOR); 07 January 2013, D.H. Costa-Rezende 115 (FLOR); 12 January 2014, L. Pereira-Silva 21 (FLOR); 12 January 2014, Pereira-Silva 22 (FLOR); 12 January 2014, L. Pereira-Silva 58 (FLOR); 12 January 2014, L. Pereira-Silva 77 (FLOR); 12 January 2014, L. Pereira-Silva 79 (FLOR); 12 January 2014, L. Pereira-Silva 77 (FLOR); 12 January 2014, L. Pereira-Silva 79 (FLOR). ARGENTINA. Jujuy: Depto Ledesma, Parque Nacional Calilegua, Abra de Cañas, 23°40'38,2"S, 64°53'46,3"O, alt. 1730 msnm, 21 May 2007, Robledo 1507 (CORD).

Additional specimens examined— BRAZIL. Amazonas: Panure, Spruce 44 (BPI 237203, isotype of Amauroderma sprucei)

*Remarks.*— The dull concentric zonate pilear surface, whitish context, a vivid orange pore surface in some specimens, small pores (5–7 [8] pores/mm), a crust with a short trichoderm in the pilear surface, the strongly dextrinoid skeletal hyphae and predominantly subglobose

spores ([7.0] 8.0–10 × 7–9  $\mu$ m), with conspicuous hollow columnar ornamentation are characteristic of this species. Decock & Herrera-Figueroa (2006, as *A. sprucei*) described the hyphal system as ditrimitic, with generative and vegetative hyphae in all parts of basidiome. Additionally, describe the trama of tubes as dimite with arboriform skeletal hyphae. In our observations, the hyphal system is considered dimitic. In the context were observed clamped generative, intercalary skeleto-biding, with long lateral and apical, thin, ramified branches, and skeletal hyphae (up to 7.0  $\mu$ m in diameter), with long portions without ramifications, tortuous, with few apical ramifications. Trama of tubes composed by clamped generative, arboriform skeletals, and thick-walled skeleto-biding hyphae, formed by a main stalk and very short lateral branches, with or without two thin apical branches.

Considering the pale context, consistence of pileus and size of pores, *A. camerarium* and *A. praetervisum* could be morphologically related, which were treated above. Both *A. camerarium* and *A. praetervisum* present conspicuous dark bands in context and distinctly larger basidiospores ([10] 11–14 [15] × [8] 9–12 [13] µm and [11.5] 12–13.5 [14] × 10–12 [13] µm, respectively). *Amauroderma schomburgkii* is also morphologically related, differing mainly due its golden brown context.

#### DISCUSSION

*Foraminisporus* is proposed here with regard to its unique endosporic ornamentation morphology and phylogenetic evidences. Despite of the low support value in LSU analyses and the differences in the topologies of BI and ML in the combined analysis, it is clear that in both phylogenetic approaches *A. sprucei* not grouped in *Amauroderma s.s.* clade, neither in *Ganoderma* clade. Additionally, in our BI analysis of ITS and BI and ML of ITS+LSU, the support of dichotomy of *Foraminisporus* from *Ganoderma/Amauroderma* is higher than those between *Ganoderma* and *Amauroderma*. In this way, the hollow columns of the endospore could be a stronger character to generic delimitation than the truncate apex, which mainly distinguishes *Ganoderma* from *Amauroderma*.

#### Ganoderma clade

The topology presented in BI of ITS+LSU is in accordance to previous works (Moncalvo et al. 1995; Moncalvo 1996; Hong & Jung 2004), corroborating the genera *Ganoderma* and *Elfvingia* P. Karst.

Considering both BI and ML analysis of LSU, except by the specimen of *G. australe* from Genbank that nested in the clade of laccate species, the result is similar to obtained in our BI ITS+LSU and those from previous works (Moncalvo et al. 1995; Moncalvo 1996; Hong & Jung 2004). The ITS topology of *Ganoderma* clade was the most incongruent considering traditional morphological features, where *H. coffeata* specimen is grouped. to *G. lucidum*. The same is observed for *A. rude* specimens.

relationship Phylogenetic of Humphreya and other Ganodermataceae genera was treated by Moncalvo (1995), which presents a topology based in the partial 25S region, showing Humphreya eminii (Henn.) Ryvarden out of the Ganodermataceae clade, "wich leads to the question of whether or not the Ganodermataceae are monophyletic". In our analysis, low support in the clade composed by G. lucidum and H. coffeata probably indicates that this relationship is not natural and the inclusion of more sequences and other molecular markers will show a different topology, which is also expected due to the morphology of pilear surface and spores of Humphreya coffeata. The most important "problem" is the presence of A. rude in this clade. In our opinion, the low support of the clade besides the morphological differences (which support the taxon in Amauroderma), may also indicate that this group is not natural. Nevertheless a similar result was observed by Glen et al. (2009), with A. rude and A. rugosum (Blume & T. Nees) Torrend grouped with Ganoderma species. Future studies are strongly recommended to observe the real relationship of the A. rude. Further information is provided in the discussion of Amauroderma s.s. clade.

#### Tomophagus clade

*Tomophagus* was proposed by Murril (as *Dendrophagus* Murrill) to accommodate *D. colossus* (Fr.) Murrill, and is characterized by a laccate and soft pileus, pale context and large ganodermoid spores. The genus was not accepted by Furtado (1965), Steyaert (1972, 1980), Corner (1983), Ryvarden (1991) and Ryvarden (2004). In our phylogenetic analysis, *Tomophagus* is not related to both *Ganoderma* and *Amauroderma*, as observed for previous works (Moncalvo et al., 1995; Moncalvo, 1996). Low support of the clade (0,66 PP value) is might due to the presence of the other taxa in the group. Neither *G.* aff. *guianensis* nor *A. brasiliense* (treated below) are morphologically related to the genus, the inclusion of more specimens of those taxa and other marker probably will present a different topology. *Ganoderma* aff.

guianensis is characterized by stipitate basidiomata, a dull and dark pilear surface, pale context with a dark cuticle, a dark crust covering the hymenophore (a continuous layer from the cuticle of pilear surface) and large, ovoid and non-truncate spores. This hymenophore crust and spores shape are quite particular, what led us to suppose that specimens are not related to *Ganoderma* or *Amauroderma*, as observed in our phylogenetic analysis as well. Further investigations are necessary to corroborate it as another genus in Ganodermataceae or not.

#### Amauroderma s.s. clade

As already observed by Moncalvo (1995, 1996), both with only one *Amauroderma* specimen, the genus was positioned as sister of *Ganoderma*, however with low statistical support. In all our BI analysis, the *Amauroderma* s.s. clade presents a high support value. Considering the species nested in this clade, is possible to note some morphological features shared by them, such as the presence of a dimitic hyphal system with clamped generative, arboriform skeletal and skeleto-binding hyphae with lateral ramifications (at least one of them in tubes or in the context), double-walled basidiospores, non-truncate and endosporic ornamentation with solid columns. Except by the morphological importance of endoporic ornamention, these features were already treated in for *Amauroderma* by Furtado (1981), Corner (1983), Moncalvo (1995), Ryvarden & Johansen (1981), Moncalvo & Ryvarden (1997), and Ryvarden (2004).

Comparing topologies of *Amauroderma s.s.* clade is quite complicated, due to the heterogeneity of distinct datasets. In a general view, the clade presents a similar topology in all analysis, mainly for those branches with high support. Considering BI of LSU and both, ML and BI of ITS+LSU, that presents the same taxa of *Amauroderma*, the topologies are quite similar. The clade composed by *A. schomburgkii*, *A. camerarium* and *A.* aff. *partitum* shares basidiomata with coriaceus to subwood consistence, while *A. intermedium* presents a distinctly soft consistence. *Amauroderma aurantiacum*, in both BI and ML analysis of ITS+LSU, nested as a sister clade of *A. schomburgkii*, *A. camerarium* and *A. partitum*, and grouped with *A. intermedium* in BI of LSU analyses. *Amauroderma aurantiacum* is the unique genus species that presents a semi-reticulate endosporic ornamentation

Similar topology was also obtained in the BI of ITS, despite inclusion of five additional taxa. The clade composed by *A*. *aurantiacum*, *A*. aff. *praetervisum* and *A*. *schomburgkii* shares a pale brown to brown context. *Amauroderma camerarium*, *A*. *partitum* and *A*. aff. *partitum* clade shares a pale context, distinct dark bands (at least in the stipe context) and coriaceus to sub-woody consistence of basidiomata. *Amauroderma calcigenum*, grouping as a sister clade of those referred above, presents thick and soft pileus, large pores and large ellipsoid spores. Further studies with additional sequences and molecular marker will probably show the true phylogenetic boundaries of the taxon. Furtado (1981) and Ryvarden (1984) considered *A. calcigenum* and *A. partitum* as synonyms, on the other hand, the phylogenetic analysis showed taxa as distinct entities, corroborating the purpose of Ryvarden (2004) and Gomes-Silva et al. (2010). Finally, *A. intermedium*, *A. pseudoboletum*, which present soft basidiomata and globose spores, grouped with *Amauroderma* sp. that is sterile and not present field observation about its consistence.

Furtado (1981) considered A. intemedium as a variety of A. rude, due to a similar microstructures in pilear surface and spores. In this context, the taxon will presents a pantropical distribution, with A. rude var. intermedium J.S. Furtado occurring in the Neotropics and A. rude var. rude in the Paleotropics. Ryvarden (2004) treated neotropical specimens as A. intermedium and suggested, such as Furtado (1981), that further studies should be carried to reveal real distribution of A. rude. In our phylogenetic studies, with Brazilian and Australian specimens, we observed two distinct clades in the ITS analyses. Despite of the position of A. rude into the Ganoderma clade with low statistical support, both terminal branches present high support value, which may indicates that they are well delimited species. As observed by Furtado (1981), the neotropical specimens present a darker pilear surface, context and tubes than A. rude, being unique morphological differences between the species. Both the phylogenetic and morphological evidences led us to believe those, A. rude and A. intermedium, are distinct species with distinct geographic occurrence.

The purpose of *Amauroderma calcitum* as a new and differently species of *A. calcigenum* and *A. partitum* by Costa-Rezende et al. (2014, Cap. I), based on morphological evidences, is here corroborate by its phylogenetic analysis. *Amauroderma* aff. *praetervisum* differs from *A. praetervisum* by presenting a grayish blue stipe in young basidiomes and smaller spores ([9.5] 10-11 [12] x 9-11  $\mu$ m vs [11.5] 12–13.5 [14] × 10–12 [13]  $\mu$ m). Morphological review of further specimens and a molecular sampling of *A. praetervisum* probably will corroborate its as a new taxon. *Amauroderma* aff. *schomburgkii* presents specimens with a remarkable ecological interaction, all of them were collected in roots of

Dicksonia sellowiana (Presl.) Hooker, differing from A. schomburgkii, presenting basidiomata with pilear surface color different, an irregular margin, and slightly larger pores [150-230 (250)  $\mu$ m vs 80-170 (180)  $\mu$ m]. Despite of the specimen of A. aff. schomburgkii appeared as a sister lineage of the main clade of A. schomburgkii (ITS+LSU and ITS analysis), phylogenetic relationship of the members in the clade is not clear yet. The inclusion of other specimens with the same morphology and ecology will probably corroborate a new taxon.

Amauroderma brasiliense clade also presents remarkable phylogenetic and morphological features. The taxa is the only known species of the genus with monomitic hyphal system in the context and dimitic with arboriform-skeletal in tubes (pers. observation), besides its soft basidiomata when fresh. Considering the literature, Singer (1983, as Scutiger brasilienses Sing.) considered the hyphal system as monomitic, Ryvarden (2004) as dimitic and Coelho et al. (2007) as monomitic to possibly dimitic, with long and thick-walled contextual hyphae sometimes resembling skeletal hyphae, but with a basal clamp. Other species of Amauroderma present a dimitic (di-trimitic or trimitic) hyphal system, both in context and tubes (Ryvarden & Johansen 1980; Furtado 1981; Moncalvo & Ryvarden 1997; Ryvarden 2004). Our phylogenetic analysis showed a lineage for this the taxon distinct and distantly of Amauroderma s.s. and Ganoderma main clade (except in ML analyses of LSU, which A. brasiliense grouped with low statistical support with the Ganoderma clade). Certainly, further studies, including more sequences and morphological review of other specimens, will corroborate it as new scientific novelties among the family.

Finally, hollow columns of the endospore are considered a new feature in Ganodermataceae studies, as well as a synapormophy for *Forominisporus*, and should be observed in the future approaches. Inclusion of other taxa as well other molecular markers are strongly encouraged for revaluation of taxonomic value of traditional characters in *Amauroderma* systematic, such as pores size, context color, pilear consistence and composition of pilear surface and all features of basidiospores.

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	Voucher			
Species	specimens	Localitie	Accesion number	
			ITS	LSU
Amauroderma				
aurantiacum	DHCR 117	Brazil	-	-
A. brasiliense	DHCR 128	Brazil	-	-
A. calcigenum	Robledo 1567	Argentina	-	*
A. camerarium	ACM 125	Brazil	-	*
A. camerarium	DHCR 96	Brazil	*	-
A. camerarium	DHCR 112	Brazil	-	-
A. camerarium	DHCR121	Brazil	*	-
A. intermedium	DS 826	Brazil	-	-
A. intermedium	MABS 321	Brazil	*	-
A. intermedium	DHCR 73	Brazil	-	*
A. intermedium	RFF 08	Brazil	-	-
A. partitum	URM 83040	Brazil	JX310854.1	*
A. partitum	URM 82884	Brazil	JX310851	*
A. partitum	URM83039	Brazil	JX310853.1	*
A. partitum	URM82882	Brazil	JX310852.1	*
A. aff. partitum	DHCR105	Brazil	-	*
A. aff. partitum	DHCR 107	Brazil	-	-
A. aff. praetervisum	DHCR 77 A	Brazil	-	*
A. pseudoboletum	Robledo 1441	Argentina	-	*
A. rude	CANB 643174	Australia	-	*
A. rude	CANB 359451	Australia	-	*
A. rude	CANB 795782	Australia	-	*
			X78753.1/X	
A. rude	J.M ASP.1	Taiwan	78774.1	*
A. schomburgkii	DHCR 69	Brazil	*	-

Table I. Species/collections/sequences used in the phylogenetic studies and their GenBank accession numbers.

# 

A. schomburgkii	DS 676 COPY	Brazil	-	-
A. schomburgkii	DHCR 66	Brazil	-	*
A. schomburgkii	DHCR 70	Brazil	-	-
A. schomburgkii	DHCR 94	Brazil	-	-
A. schomburgkii	DHCR 95	Brazil	-	-
A. schomburgkii	DHCR 97	Brazil	*	-
A. schomburgkii	DHCR 99	Brazil	-	-
A. schomburgkii	DHCR 100	Brazil	-	-
A. schomburgkii	CHAP 24	Brazil	-	*
A. aff. schomburgkii	DHCR 118	Brazil	-	-
A. sprucei	DHCR 113	Brazil	-	-
A. sprucei	DHCR 114	Brazil	-	*
A. sprucei	DHCR 115	Brazil	*	-
A. sprucei	ACM 534	Brazil	-	*
Amauroderma sp.	INPA 249751	Brazil	-	*
Ganoderma aff.	GAS 076	Drozil		*
guianensis C. off. mimmin	0AS 070	Diazii	-	*
G. all. guianensis	DHCK 12/	Brazii	- X78750 1/X	×78780
G. australe	RSH 705	Taiwan	78771.1	.1
			X78749.1/X	X78777
G. boninense	RS	Taiwan	78770.1	.1
G. cf. australe	MABS 303	Brazil	-	-
G. cf. australe	Mat. BARUFFI	Brazil	-	*
G. cf. australe	ACM 418	Brazil	-	-
G. cf. australe	JM FLO 8	Brazil	*	-
G. lobatum	BAFC2411	Argentina	AF169989.1	*
G. lobatum	BAFC2391	Argentina	AF169991.1	*
	R7		X78743.1/X	X78776
G. lucidum	R2	Taiwan	78764.1	.1
G. lucidum	Dai2272	Sweden	JQ781851.1	*
G. lucidum	Dai 11593	Finland	JQ781852.1	*
G. microsporum	RSH 821	Taiwan	X78751.1/X	X78779

		78772.1	.1
		X78746.1/X	X78778
J2	Japan	78767.1	.1
DHCR 65	Brazil	*	-
JS 42	Brazil	-	*
			FJ3938
MUCL 49758	Argentina	FJ411085.1	57
			FJ3938
MUCL 47129	Argentina	FJ411082	54
	J2 DHCR 65 JS 42 MUCL 49758 MUCL 47129	J2JapanDHCR 65BrazilJS 42BrazilMUCL 49758ArgentinaMUCL 47129Argentina	78772.1   X78746.1/X   J2 Japan   DHCR 65 Brazil   JS 42 Brazil   MUCL 49758 Argentina   FJ411085.1   MUCL 47129 Argentina

- Sequence to be deposited; \* No sequence

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Figure 1. Phylogenetic tree from Bayesian inference of ITS dataset. Numerals on branches are posterior probabilities (PP < 0.70 are not showed), branches in bold present full PP value.



0.02

Figure 2. Phylogenetic tree from Bayesian inference of LSU dataset. Maximum likelihood presents a similar topology (divergences are marked with a \* and discussed in text). Numerals on branches are posterior probabilities (before slash markers; PP < 0.70 are not showed) and bootstrap probabilities (after slash markers; BP < 70 are not shown), branches in bold present full PP or bootstrap value.



Figure 3. Phylogenetic tree from Bayesian inference of ITS+LSU dataset. Numerals on branches are posterior probabilities (PP < 0,70 are not showed), branches in bold present full PP value.


Figure 4. Phylogenetic tree from Maximum Likelyhood inference of ITS+LSU dataset. Numerals on branches are bootstrap value (BP<70% are not showed), branches in bold present full bootstrap value.





Figure 5. Basidiospores of *Foraminisporus sprucei*. A,B- optical microscopy (KOH and Cotton blue, respectively). C-F SEM micrographs. C- general view showing holes (of hollow columns) out side of exospore. D, E, F- general view of total or partial remotion of exospore wall showing hollow collumns of the endospore. E, D- arrow indicates the hollow collumn in detail. Scale bar: A, B- 10  $\mu$ m; C- 2  $\mu$ m; D, E, F- 1  $\mu$ m.



Figure 6. Basidiospores of *Amauroderma calcigenum*. A,B- optical microscopy (KOH and Cotton blue, respectively). C-F SEM micrographs. C- general view showing exospore without holes. D- side view of broken basidiospore showing the collumns reaching the exospore and smaller secondary projections. E, F-general view, with total exospore remotion, showing solid collumns and smaller secondary ornamentation. Scale bar: A- 10  $\mu$ m; B- 5  $\mu$ m; C - 1  $\mu$ m; D, F- 2  $\mu$ m; E- 10  $\mu$ m.



Figure 7. Basidiospores of *Amauroderma pseudoboletum*. A, B- optical microscopy (KOH and Cotton blue, respectively). C-F SEM micrographs. C-general view showing exospore without holes. D- side view of broken basidiospore, showing the collumns reaching the exospore and smaller secondary projections. E- general view, with total exospore remotion, showing solid collumns and smaller secondary ornamentation of the endospore. F- Detail in solid collumns and smaller secondary ornamentation of the endospore. Scale bar: A- 10  $\mu$ m; B- 5  $\mu$ m; C, D - 1  $\mu$ m; E, F- 2  $\mu$ m.





Figure 8. Basidiospores of *Amauroderma schomburgkii*. A, B- optical microscopy (KOH and Cotton blue, respectively). C-F SEM micrographs. C-general view showing exospore without holes. D- side view of broken basidiospore, showing the collumns reaching the exospore and smaller secondary projections. E- general view, with partial exospore remotion, showing solid collumns and smaller secondary ornamentation of the endospore. F- general view, with total exospore remotion, showing solid collumns and smaller secondary ornamentation of the endospore. F- general view, with total exospore remotion, showing solid collumns and smaller secondary ornamentation of the endospore. Scale bar: A- 10  $\mu$ m; B, C- 5  $\mu$ m; D, E, F- 1  $\mu$ m.

## 6. CONCLUSÃO E RECOMENDAÇÕES PARA TRABALHOS FUTUROS

- Amauroderma apresenta, até o momento, cerca de 30 espécies aceitas, no entanto coleções de espécies publicadas para a China devem ser criticamente revisadas, visto que existem algumas divergências na literatura, podendo alterar significativamente a diversidade do gênero. Neste trabalho são apresentadas duas novas espécies e uma sinonímia.

- A partir de análises morfológicas detalhadas (macro, micro e ultraestrutural) e filogenéticas um gênero novo é apresentando, trazendo a tona, um novo caracter na sistemática de Ganodermataceae, que deve ser observados em espécies que não foram apresentadas nesse trabalho.

- Estudos posteriores provavelmente irão corroborar dois novos táxons citados como *affinis* no capítulo 3. No caso de *A. aff. schomburgkii* é possível observar a relação específica com *Dicksonia sellowiana*, podendo ser a primeira citação de relação de especificidade de hospedeiro para o gênero, apontando a necessidade desse tipo de estudo em trabalhos futuros.

- Amauroderma brasiliense é a única espécie do gênero que apresenta contexto monomítico e apresentou-se em todas as análises fora do clado de Amauroderma, podendo ser corroborada em estudos posteriores como um novo gênero. Os materiais tratados como Ganoderma aff. guianensis apresentam basidiomas estipitados, esporos ovoides e não truncados, diferindo tanto dos conceitos genéricos de Amauroderma quanto dos outros gêneros de Ganodermataceae, adicionalmente, os espécimes agruparam-se em um clado fora de Amauroderma e Ganoderma, podendo também representar um novo gênero.

- Algumas outras espécies do gênero que apresentam morfologia diferente da apresentada aqui (capítulo III), por exemplo, *A. coltricioides* Henkel, Aime & Ryvarden (esporos lisos), *A. deviatum* Ryvarden (esporos com ornamentação "honey-comb"), *A. andinum* Ryvarden (basidiomas sésseis) devem ser abordadas filogeneticamente, com o intuito de construir um conceito natural de *Amauroderma*. Análises filogenéticas *multiloci* com espécimes de várias regiões são extremamente necessárias para apontar a real significância de caracteres usualmente utilizados na sistemática do gênero, como consistência, coloração do contexto e microestruturas da superfície do píleo, entre outras características dos esporos. - Como já esperado, quase todos os espécimes coletados para este trabalho (exceto um) estavam associados a raízes de plantas, mostra-se a necessidade de que estudos futuros comprovem qual é o tipo de relação entre as espécies de *Amauroderma* e os substratos vegetais com os quais se associam.

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Tabela I. Lista de exsicatas revisadas

Táxon	Herbário e nº de acesso
Amauroderma aurantiacum	LPS 003 (FLOR)
A. aurantiacum	LPS 004 (FLOR)
A. aurantiacum	LPS 008 (FLOR)
A. aurantiacum	LPS 012 (FLOR)
A. aurantiacum	LPS 014 (FLOR)
A. aurantiacum	LPS 017 (FLOR)
A. aurantiacum	LPS 025 (FLOR)
A. aurantiacum	GAS 077 (FLOR)
A. aurantiacum	DHCR 030 (FLOR)
A. aurantiacum	LPS 002 (FLOR)
A. brasiliense	DHCR 128 (FLOR)
A. brockesii	BPI 302396
A. calcigenum	BPI237146
A. calcigenum	URM 80725
A. calcigenum	URM 80661
A. calcigenum	URM 80634
A. calcigenum	URM 82777
A. calcigenum	Robledo 1567 (CORD)
A. calcigenum	Robledo 1875 (CORD)
A. calcitum	LPS 069 (FLOR)
A. calcitum	DHCR 104 (FLOR)
A. calcitum	DHCR 105 (FLOR)
A. calcitum	DHCR 130 (FLOR)
A. calcitum	DHCR 031 (FLOR)
A. calcitum	LPS 015 (FLOR)
A. calcitum	LPS 019 (FLOR)
A. calcitum	LPS 016 (FLOR)

A. calcitum	LPS 007 (FLOR)
A. calcitum	LPS 081 (FLOR)
A. calcitum	GAS 549 (FLOR)
A. calcitum	DHCR 110 (FLOR)
A. calcitum	LPS 009 (FLOR)
A. calcitum	DHCR 106 (FLOR)
A. calcitum	DHCR 107 (FLOR)
A. calcitum	GAS 072 (FLOR)
A. calcitum	GAS 071 (FLOR)
A. calcitum	GAS 074 (FLOR)
A. calcitum	DHCR 111 (FLOR)
A. camerarium	GAS S/N (FLOR)
A. camerarium	GAS 090 (FLOR)
A. camerarium	GAS 131 (FLOR)
A. camerarium	GAS 171 (FLOR)
A. camerarium	GAS 172 (FLOR)
A. camerarium	GAS 198 (FLOR)
A. camerarium	GAS 475 (FLOR)
A. camerarium	DHCR 112 (FLOR)
A. camerarium	BPI 327148
A. camerarium	SP 61451
A. camerarium	DHCR 121 (FLOR)
A. camerarium	UEFS 106068
A. camerarium	UEFS 133888
A. cf. camerarium	URM 83424
A. cf. praetervisum	URM 83577
A. corneri	SP 213543
A. elegantissimum	URM 82788
A. elegantissimum	URM 81026

A. elegantissimum	JPB 49380
A. exile	K 180667
A. exile	URM 83411
A. exile	JPB 49374
A. exile	JPB 49376
A. heteromorphum	BPI 237166
A. intermedium	DHCR 73 (FLOR)
A. intermedium	DS 826 (FLOR)
A. intermedium	MABS 321 (FLOR)
A. intermedium	RFF 008 (FLOR)
A. omphalodes	NY 730851
A. opacaespitosa	Robledo 499 (CORD)
A. opacaespitosa	Robledo 484 (CORD)
A. opacaespitosa	Robledo 1086 (CORD)
A. partitum	K 181861
A. partitum	URM 82872
A. partitum	FLOR 4554
A. partitum	FLOR 44756
A. partitum	FLOR 48461
A. partitum	CS 022 (FLOR)
A. partitum	JPB 6856
A. partitum	JPB 9241
A. partitum	JPB 7256
A. praetervisum	LPS 013 (FLOR)
A. praetervisum	LPS 066 (FLOR)
A. praetervisum	LPS 067 (FLOR)
A. praetervisum	LPS 068 (FLOR)
A. praetervisum	BPI 237178
A. praetervisum	BPI 237179

A. praetervisum
A. praetervisum
A. praetervisum
A. praetervisum
A. aff. praetervisum
A. aff. praetervisum
A. aff. praetervisum
A. pseudoboletum
A. pseudoboletum
A. pseudoboletum
A. pseudoboletum
A. rude
A. rude
A. rude
A. schomburgkii

BPI 747518 BPI 237193 URM 79293 JPB 5860 **URM 84230** DHCR 77 (FLOR) DHCR 79 (FLOR) SP 61135 Robledo 1253 (CORD) Robledo 1441 (CORD) BPI 237180 CANB 359451 CANB 643174 CANB 795782 GAS 393 (FLOR) GAS 401 (FLOR) BPI 747459 BPI 747390 BPI 237122 BPI 237192 **UEFS 132218** DHCR 118 (FLOR) DHCR 094 (FLOR) DHCR 099 (FLOR) DHCR 066 (FLOR) DHCR 095 (FLOR) DHCR 100 (FLOR) DHCR 070 (FLOR) JPB 49388

A. schomburgkii	JPB 49390
A. schomburgkii	JPB 6857
A. schomburgkii	JPB 8369
A. schomburgkii	JPB 8374
A. schomburgkii	JPB 7265
A. sprucei	DHCR 113 (FLOR)
A. sprucei	DHCR 114 (FLOR)
A. sprucei	DHCR 115 (FLOR)
A. sprucei	LPS 21 (FLOR)
A. sprucei	LPS 22 (FLOR)
A. sprucei	LPS 58 (FLOR)
A. sprucei	LPS 77 (FLOR)
A. sprucei	LPS 79 (FLOR)
A. sprucei	BPI 237203
A. sprucei	Robledo 1507 (CORD)
A. sprucei	URM 82827
A. sprucei	URM 82084
A. torrendii	BPI 307270
Amauroderma sp.	INPA 249751
Ganoderma sp.	DHCR 065 (FLOR)
G. aff. guianenses	DHCR 127 (FLOR)
G. aff. guianenses	GAS 076 (FLOR)
G. cf. australe	MABS 303 (FLOR)
G. cf. australe	ACM 418 (FLOR)
G. cf. australe	JM (FLO) 08 (FLOR)
Haddowia longipes	CANB 629042
Humphreya coffeata	JS 042
Polyporus ocellatus	BPI 215326