

Article



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Amauroderma calcitum sp. nov. and notes on taxonomy and distribution of Amauroderma species (Ganodermataceae)

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Abstract

During a polypore survey in the Cerrado (Mato Grosso state, Brazil), *Amauroderma* specimens were collected and some of them deserved special taxonomic attention due to their intermediate morphology between *A. calcigenum* and *A. partitum*. A comparative study of the types and reference material, as well as phylogenetic analysis, led us to conclude that these specimens represent an undescribed species, presented here as *Amauroderma calcitum*. In addition *A. brasiliense* is presented as a new record for the Brazilian Cerrado. Illustrations, taxonomic analyses and a discussion are presented for each taxon, and a key to the *Amauroderma* species from Brazil is provided. A phylogenetic discussion about the genus is also presented.

Key words: Molecular phylogeny, Neotropics, Polyporales, systematics

Introduction

The polypore genus *Amauroderma* Murrill (1905: 366) comprises usually stipitate species found associated with fallen dead wood or roots of living or dead trees with a tropical and subtropical distribution (Furtado 1981, Ryvarden 2004). The basidiospores are globose to ellipsoid, non-truncated, double-walled with an ornamented inner layer [rarely smooth as in *A. coltricioides* T.W. Henkel, Aime & Ryvarden in Aime *et al.* (2003: 615)].

There have been several taxonomic studies on Brazilian species of *Amauroderma* over the past 10 years (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). Gomes-Silva *et al.* (2015) presented the genus as non-monophyletic and produced a clade composed of Brazilian specimens including specimens of *A. schomburgkii* [= *Fomes regulicolor* Berk. ex Cooke (1886: 21)], the type species of the genus, which might be interpreted as *Amauroderma stricto sensu*, while the remaining clades may represent new genera.

Knowledge about the genus in the Cerrado (Brazilian savannah) is restricted to original records of seven species [i.e. *A. aurantiacum* (Torrend) Gibertoni & Bernicchia (2008: 322), *A. calcigenum* (Berk.) Torrend (1920: 129), *A. omphalodes* (Berk.) Torrend (1920: 131), *A. praetervisum* (Pat.) Torrend (1920: 131), *A. rude* (Berk.) Torrend (1920: 127) and *A. schomburgkii* (Mont. & Berk.) Torrend (1920: 140) and *A. sprucei* (Pat.) Torrend (1920: 121), as *A. dubiopansum* (Lloyd) Ryvarden (2004: 52)], that are later cited in regional checklists (Gibertoni & Drechsler-Santos 2010, Abrahão *et al.* 2012 and Gugliotta *et al.* 2015).

During the study of *Amauroderma* from Cerrado, central Brazil, some specimens were found that had interesting, intermediate morphologies between *A. calcigenum* and *A. partitum* (Berk.) Wakef. (1934:242). The taxonomic status

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of *A. calcigenum* and *A. partitum* has been discussed several times in the specialized literature, because these taxa have been considered as synonyms (Furtado 1981, Ryvarden 1984) or as distinct species (Ryvarden 2004, Gomes-Silva *et al.* 2010, Gomes-Silva *et al.* 2015).

The detailed examination and comparison with reference materials and molecular analyses indicated that those specimens intermediate to *A. calcigenum* and *A. partitum* represent a new species, which is described here. Additionally, we present comments on other *Amauroderma* species that occur in the Cerrado, a phylogenetic discussion of the genus and a key to the species reported from Brazil.

Materials & Methods

Sampling and morphological studies

Samples were collected from 2011 to 2014 in the Chapada dos Guimarães National Park (15°10'S–15°30'S and 55°45'W–56°00'W), Mato Grosso state, in the Brazilian Cerrado. The Cerrado is located in the central region of Brazil, and is characterized by a typical savannah vegetation type, with a marked seasonal climate with rainy summers and dry winters (Aguiar & Camargo 2004, Felfilli *et al.* 2000). The words Amazonia, Atlantic Rain Forest, Caatinga and Cerrado are used here as phytogeographic domains (Andrade-Lima 1981, ter Steege *et al.* 2003, Stehmann *et al.* 2009, Batalha 2011). The basidiomata were labeled and stored in paper bags in the field, then taken to the laboratory and dried at 38°C.

Colors are described according to Munsell (1975). For the study of the hyphal system, sections of the basidiomata were incubated in hot (40°C) 3% NaOH solution, then dissected under a stereomicroscope and finally examined in 3% NaOH solution at room temperature (Teixeira 1995). Specimens were also examined in Melzer's reagent and 4% KOH (Ryvarden 2004). All microscopic measurements were carried out in Melzer's reagent. In presenting the size range of pores, hyphae and basidiospores, 5% of the measurements at each end of the range are given in parentheses, when relevant. In the text, the following abbreviations are used: ave = arithmetic mean, Q = the ratio of length/width of basidiospores and ave-Q = arithmetic mean of the ratio Q (Amalfi $et\ al.\ 2010$). We follow the Resupinate Russulales species database (http://www.cbs.knaw.nl/russulales/) to define the terminology of the basidiospore shape based on the Q value and symmetry. The terminology used for describing the pileipellis follows Furtado (1981, as pilear cover).

Boxplots based on length and width of basidiospores from *A. calcigenum* [URM80634, URM80661, URM 80725, FLOR 52315, FLOR 52316, BPI 237146, BPI 302396, BPI 307270, (n = 280/8)], *A. partitum* [FLOR 45554, FLOR 44756, FLOR 48461 (n = 120/3)] and the new species [SP 102719, FLOR 52228, FLOR 52238, FLOR 52239, FLOR 52239, FLOR 52232, FLOR 52234, FLOR 52243, FLOR52230, (n = 280/8)] were generated in "R" software (R Development Core Team 2011). A multivariate ordination method [Non-metric multidimensional scaling (NMDS)] (Taguchi & Oono 2005) was performed in the software Past (Hammer *et al.* 2001), based on means of morphological characters, such as basidiospore size, pore diameter, dissepiment thickness, pores per millimeter, microstructures of the pilear surface, pileus consistency and thickness from *A. calcigenum* (n = 8), *A. partitum* (n = 4) and the new species (n = 8) with the same specimens used to construct the boxplots (including K810681 for *A. partitum*). Specimens collected during this work are kept at the herbarium FLOR. Reference materials used for morphological comparison are listed in Table 1. Herbarium acronyms follow Thiers B. [continuously updated].

TABLE 1. Species, vouchers and localities of specimens used for morphological comparison.

Species	Voucher	Localitie	
A. brasiliense	SP 213543 (Type of A.corneri)	Brazil	
A. calcigenum	BPI 237146 (Isotype of Polyporus calcigenus)	Brazil	
	BPI 302396 (Type of P. brockesii)	Brazil	
	BPI 307270 (Type of P. torrendii)	Brazil	
	URM 80725	Brazil	
	URM 80661	Brazil	
	URM 80634	Brazil	
	URM 82777	Brazil	

TABLE 1. (Continued)

Species	Voucher	Localitie
A. pseudoboletus	FLOR 52315	Argentina
	FLOR 52316	Argentina
A. camerarium	BPI 327148 (Syntype)	Brazil
	SP 61451	Brazil
A. exile	K 180667 (Isotype)	Brazil
	URM 83411	Brazil
A. omphalodes	NY 730851 (Type of P. omphalodes)	Brazil
A. partitum	K 181861 (Syntype)	Brazil
	URM 82872	Brazil
	FLOR 45554	Brazil
	FLOR 44756	Brazil
	FLOR 48461	Brazil
A. praetervisum	BPI 23178	Brazil
	BPI 237179	Colombia
	BPI 237193	Colombia
	BPI 747518	Costa Rica
A. pseudoboletus	SP 61135	Brazil
	FLOR52315	Argentina
A. schomburgkii	BPI 215326 (Isotype of P. ocellatus)	Brazil
	BPI 237166 (Isotype of <i>P. heteromorphum</i>)	French Guiana
	BPI 747459	French Guiana
	BPI 747390	Costa Rica
	BPI 237192	Costa Rica
	BPI 237122	Panama
A. sprucei	BPI 237203 (Isotype)	Brazil

Genomic DNA extraction, PCR and Sequencing

Extraction of total genomic DNA from dried basidiomata followed the protocol of Doyle & Doyle (1987) modified by Góes-Neto *et al.* (2005). Primer pairs ITS8-F/ITS6-R (Dentinger *et al.* 2010) and LR0R/LR7 (Vilgalys & Hester 1990) were used to amplify the nrITS and nrLSU regions respectively, by a qualitative simplex polymerase chain reaction. Sanger Sequencing was performed with *BigDye Terminator v.*3.1 *Cycle Sequencing Kit* (Applied Biosystems, California, USA) following manufacturer procedures using the same oligos as forward and reverse sequencing primers for the nrITS, and replacing the LR7 by the LR5 as reverse primer for the nrLSU. The sequencing was performed at FIOCRUZ-MG (Brazil), as part of the FungiBrBol project. The newly generated sequences and additional sequences downloaded from GenBank are listed in Table 2.

TABLE 2 Species, vouchers and accession numbers used in phylogenetic analyses.

Species name	Voucher	Genbank - ITS/LSU	
A. aurantiacum	FLOR 52205	KR816510/KU315205	
A. aurantiacum	URM 78847	JX310840/-	
A. brasiliense	FLOR 50932	KU315196/KU315206	
A. brasiliense	TBG 58	JX982569/-	
A. brasiliense	URM 83578	JX310841/-	
A. calcigenum	FLOR 52315	KR816514/-	

TABLE 2. (Continued)

Species name	Voucher	Genbank - ITS/LSU
4. calcigenum	URM 83864	JX982565/-
4. calcitum	FLOR 50931	KR816528/KU315207
4. calcitum	FLOR 52230	KR816529/-
4. elegantissimum	URM 82790	JX310842/-
4. elegantissimum	URM 82789	JX310844/KT006617
4. elegantissimum	URM 82787	JX310843/KT006616
4. exile	URM 82794	JX310845/-
4. exile	HFSL (ACGS41A)	-/KT006622
A. floriformum	URM83250	JX310846/-
4. intermedium	FLOR 52246	KR816524/KU315208
A. intermedium	FLOR 52979	KR816526/-
A. intermedium	FLOR 52248	KR816527/KU315209
A. laccatostiptatum	URM 83238	JX310847/-
A. omphalodes	FLOR 52216	KR816509/-
A. omphalodes	FLOR 52169	KR816523/-
A. omphalodes (as A. sprucei)	URM 83908	JX982568/-
A. omphalodes (as A. sprucei)	URM 84236	KC348462/-
1. partitum	URM 82884	JX310851/-
1. partitum	URM 83039	JX310853/-
1. partitum	URM 83040	JX310854/-
1. partitum	URM 82882	JX310852/-
A. partitum	URM 83229	JX310849/-
A. partitum	URM 83233	JX310850/-
4. partitum	URM 83229	-/KT006619
4. partitum	URM 83233	-/KT006620
A. aff praetervisum	FLOR 52249	KR816511/-
A. praetervisum	URM 84223	KC348460/-
4. praetervisum	REC18707	JX310855/-
4. praetervisum	URM 84230	KC348461/-
4. praetervisum	GOMES SILVA 909	JX310856/-
A. pseudoboletus	FLOR 52318	KR816516/-
4. rude	CANB 643174	KU315197/-
4. rude	CANB 795782	KU315198/-
4. rude	CANB 359451	KU315199/-
4. rude var. intermedium	JMM ASP	X78753&X78774/-
4. rugosum	THP 318	HM480836/-
4. rugosum	THP 30	HM480835/-
4. aff schomburgkii	FLOR 52304	KR816521/-
4. schomburgkii	FLOR 50929	KR816520/KU315210
4. schomburgkii	FLOR 39372	KR816519/KU315211
4. schomburgkii	URM 84254	KC348459/-
A. schomburgkii	FLOR 38272	KR816512/-

TABLE 2. (Continued)

Species name	Voucher	Genbank - ITS/LSU
A. schomburgkii	FLOR 39388	KR816513/KU315212
4. schomburgkii	FLOR 50930	KR816515/KU315213
4. schomburgkii	FLOR 39122	KR816517/KU315214
4. schomburgkii	FLOR 52176	KR816518/-
4. schomburgkii	FLOR 52177	KR816522/KU315215
4. schomburgkii	URM 83902	JX982571/-
A. schomburgkii	URM 82829	JX310857/-
4. schomburgkii	HFSL (ACGS944)	JX310858/-
4. schomburgkii	URM 83904	JX982567/-
4. schomburgkii	URM 84214	KC348455/-
4. schomburgkii	URM 84226	KC348456/-
4. schomburgkii	URM 83228	JX310848/KT006621
4. <i>sp</i>	INPA 249751	KR816525/-
4. <i>sp</i>	THP 26	HM480834/-
4. sp	BRFM 915	JX082335/-
4. sprucei	FLOR 52191	KU315200/KU315216
4. sprucei	FLOR 52184	KU315201/-
4. sprucei	FLOR 52195	KU315202/-
1. subresinosum	THP 16	FJ154782/-
1. subresinosum	ML 288	JQ409358/-
1. subresinosum	FRIM 95	AJ627583/-
4. subssessile (as A. sessile)	URM 83905	JX982570/-
1. subssessile	URM83239	JX310860/-
Ganoderma applanatum (Pers.) Pat. (1889:	JM 97/56	AF255099/-
67) cplx		
G. applanatum cplx	JM 98/132	AF255115/-
G. applanatum cplx	ME-GAN-14	AF255130/-
G. applanatum	Dai 12483	KF494999/KF495009
G. applanatum cplx	RV-PR10	AF255133/-
G. australe (Fr.) Pat. (1889: 71) cplx	BAFC 2531	AF255176/-
G. australe cplx	FLOR 52289	KU315203/KU315217
G. australe cplx	PKB 91/098	AF255163/-
G. australe cplx	BAFC 671	AF255184/-
G. australe cplx	BAFC 1544	AF255185/-
G. australe	Cui 9511	JN048773/JN048792
G. australe	URM 83325	JQ514106/-
G. australe	RSH 0705	X78750&X78771/X78780
G. boninense Pattouillard (1889: 72)	RSH RS	X78749&X78770/X78777
G. coffeatum (Berk.) J.S. Furtado (1967: 383)	FLOR 50933	KU315204/-
G. lingzhi S.H. Wu, Y. Cao & Y.C. Dai (2012: 54)	WD-565	EU021455/-
G. lingzhi	WD-2038	EU021456/-

TABLE 2. (Continued)

Species name	Voucher	Genbank - ITS/LSU
G. lucidum (Curtis) P. Karst.(1881: 17)	K 175217	KJ143911/-
G. lucidum	RZ	X78743&X78764/X78776
G. lucidum	BR 4195	KJ143909/-
G. microsporum R.S. Hseu (1989: 36)	0821	X78751&X78772/X78779
G. multipileum Ding Hou (1950: 101)	CWB 01740	EU021461/-
G. multipileum	BCRC 37033	EU021462/-
G. multiplicatum(Mont.) Patouillard (1889: 74)	URM 83346	JX310823/JX310837
G. orbiforme (Fr.) Ryvarden (2000: 187)	URM 83332	JX310813/JX310827
G. orbiforme	URM 83334	JX310814/JX310828
G. orbiforme	URM 83335	JX310815/JX310829
G. orbiforme	URM 83336	JX310816/JX310830
G. parvulum Murrill (1902: 605)	URM 83339	JX310817/JX310831
G. parvulum	URM 2948	JX310821/JX310835
G. parvulum	URM 83340	JX310818/JX310832
G. parvulum	URM 80765	JX310822/JX310836
G. resinaceum Boud in Pattouillard (1889:72)	BCRC 36147	KJ143916/-
G. sinense J.D. Zhao, L.W. Hsu & X.Q. Zhang(1979: 272)	Wei 5327	KF494998/KF495008
G. tsugae Murrill (1902: 601)	J2	X78746& X78767/X78778
Pe. chaquenia Robledo & Decock in Robledo et al. (2009:662)	MUCL 47647	FJ411083/FJ393855
Pe. pendula Decock & Ryvarden (2003: 99)	MUCL 46034	FJ411081/FJ393853
T. cattienensis Le Xuan Tham & J.M. Moncalvo in Tham et al. (2012: 777)	CT 99	JN184397/-
T. cattienensis	CT 119	JN184398/-
T. colossus (Fr.) Murrill (1905b: 197)	URM 80450	JX310825/JX310839
T. colossus	ANH s.n.	JN184395/-
T. colossus	HCMC 10	JN184396/-
T. colossus	URM 83330	JQ618247/JX310811

Phylogenetic analyses

Sequences were manually edited using Genious v. 6.1.8 (http://www.geneious.com, Kearse *et al.* 2012). Sequences of nrITS and nrLSU of Ganodermataceae and outgroups [*Perenniporiella chaquenia* Robledo & Decock (2009: 662), *Pe. pendula* Decock & Ryvarden (2003: 99)] were retrieved from GenBank (NCBI) and combined with the new sequences to construct two datasets, one composed only of nrITS sequences and the other combining nrITS+ nrLSU sequences. In order to corroborate the delimitation of the new species proposed here in the combined dataset, and due to the lack of sequences from the same collection, sequences of *A. exile* and *A. partitum* from different vouchers (but from the same locality) studied by Gomes-Silva *et al.* (2015) were used in the analyses. The datasets were aligned using MAFFT v.7 (Katoh & Standley, 2013), under the G-INS-1 criteria, then manually inspected using MEGA v.6 (Tamura *et al.* 2013). The indels present in nrITS were recorded as binary characters following the simple indel coding method (SIC, Simmons & Ochoterena 2000) as implemented in the SeqState software (Müller 2005). The nrITS dataset was subdivided into three data partitions, ITS1, 5.8S and ITS2, while the combined was subdivided in four: ITS1, 5.8S, ITS2 and LSU.

The best fit model of nucleotide evolution to the dataset was selected with AIC (Akaike Information Criterion) using jModelTest2 v.1.6 (Darriba *et al.* 2012; Guindon & Gascuel, 2003). Bayesian Inference (BI) and Maximum

Likelihood (ML) phylogenetic analyses were applied to the datasets. BI was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with two independent runs, each one beginning from random trees with four simultaneous independent chains, performing 1x10⁷ replications, sampling one tree every 1x10³th generation. The first 2500 sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a 50% majority-rule consensus tree and calculate Bayesian posterior probabilities (BPP) of the clades. ML searches were conducted with RAxML-HPC v.8.2.3 (Stamatakis 2014), available in the CIPRES science gateway (Miller *et al.* 2010, http://www.phylo.org/). The analysis first involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree, under a GTRGAMMAI model, with all other parameters estimated by the software. Only the best scored ML tree from all searches was kept. To assess the reliability of the nodes, multiparametric bootstraping replicates under the same model were computed to allow the program to halt bootstrapping automatically by the autoMRE option. We provided an additional alignment partition file to force RAxML software to search for a separate evolution model for each dataset including the recoded indels.

A node was considered to be strongly supported if it showed a BPP \geq 0.95 and/or BS \geq 90%, while moderate support was considered when BPP \geq 0.9 and/or BS \geq 70%. The final alignment, as well the resulted topologies, were deposited in TreeBASE (http://www.treebase.org/treebase/index.html), under accession ID:18616 (http://purl.org/phylo/treebase/phylows/study/TB2:S18616).

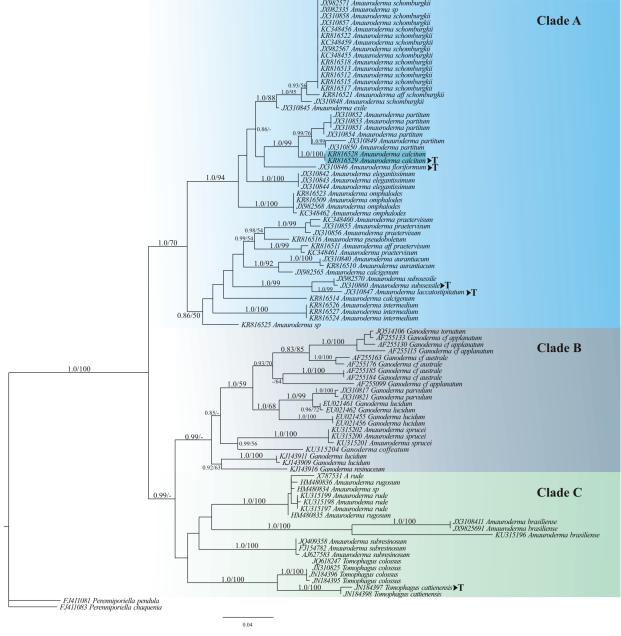


FIGURE 1. Maximum likelihood (ML) tree of *Amauroderma* and related genera from dataset of 94 ITS sequences. Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown. **T** = type.

Results

Phylogenetic analyses

The final nrITS alignment contains 94 sequences, 738 characters including gaps, of which 386 are constant, 352 variable and 278 parsimony informative. The combined (nrITS + nrLSU) dataset contains 41 sequences, with 1603 characters including gaps, in which 1233 are constant, 370 variable and 257 parsimony informative. The evolutionary models selected for nrITS dataset were TrNef+I+G, JC, TPM2uf+G for ITS1, 5.8S and ITS2, respectively. For the combined dataset the models were TIM1+G (ITS1), JC (5.8S), TPM3+I+G (ITS2) and TIM1+I+G(nrLSU). ML and BI analyses generated trees with identical topologies. Only the best scored ML trees for each dataset are shown (Figs. 1, 2).

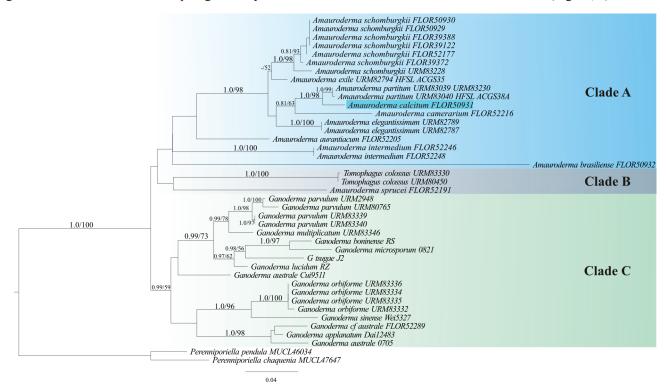


FIGURE 2. Maximum likelihood (ML) tree of *Amauroderma* and related genera from dataset of 41 ITS+LSU sequences. Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown.

The nrITS topology shows three main clades. The first (clade A), is well supported by BI (1.0 BPP) and moderately supported in ML analyses (70% BS), and is composed only by neotropical *Amauroderma* species. Within this clade, the new species formed a well-supported terminal branch (100% BS, 1.00 BPP), clustering as sister clade (98% BS, 1.00 BPP) to *A. partitum*. The second (clade B), well supported by BI analyses (0.99 BPP), but weakly supported in ML analyses, is composed of dull and laccate *Ganoderma* P. Karst. (1881: 17) species, as well as *Amauroderma sprucei* (Pat.) Torrend (1920: 121). The third clade (clade C) is not significantly supported and is composed of *Amauroderma* specimens from Asia and Oceania, *A. brasiliense* (Singer) Ryvarden (2004: 44) from the Neotropics, and *Tomophagus* Murrill (1905b: 197) species.

The combined dataset also presented three main clades. The first, (clade A) is non-supported and is composed of neotropical *Amauroderma* species. The second one (clade B) is non-supported and is composed of *T. colossus* (Fr.) Murrill (1905b: 197) and *A. sprucei*. The third one (clade C) was recovered with no significant support in ML (59% BS), but well-supported in BI (0.99 BPP) and is composed of dull and laccate *Ganoderma* species.

Non-metric multidimensional scaling (NMDS)

The NMDS plot (Fig. 6) presents three clearly distinct groups, each of them specifically composed of specimens of *A. calcigenum*, *A. partitum* and the new species (stress 0.13).

Taxonomy

Fifty nine specimens representing seven species were collected during this work. Among them a new species is proposed and *A. brasiliense* is reported for the first time from the Cerrado.

Amauroderma calcitum Costa-Rezende & Drechsler-Santos *sp. nov*. Fig. 3 a–c, 8 c Mycobank:—812846.

Diagnosis:—Basidiome stipitate, pileus up to 6.0 mm thick, with a reddish brown, slightly shiny pilear surface, pale context, brown and slightly velvety stipe, large lacerate pores, hyphal system dimitic and pale yellowish, broadly ellipsoid to ellipsoid basidiospores [11–14 (15) × (7) 8–10.5 (11) μm]. Similar to *A. calcigenum* and *A. partitum*; differing from *A. calcigenum* by its narrower basidiospores [(7) 8–10.5 (11) μm vs 10–12 (12.5) μm] and coriaceus pileus, and from *A. partitum* by presenting a thicker pileus (2.5–6.0 mm vs 0.9–1.1 mm thick).

Etymology:—"calcitum" is a neologism referring to its intermediate morphology when compared to A. calcigenum and A. partitum.Holotype:—BRAZIL. MATO GROSSO: Chapada dos Guimarães, Chapada dos Guimarães National Park, 07 Jan 2013, D.H. Costa-Rezende 105 (FLOR52230). Isotype in HUEFS.

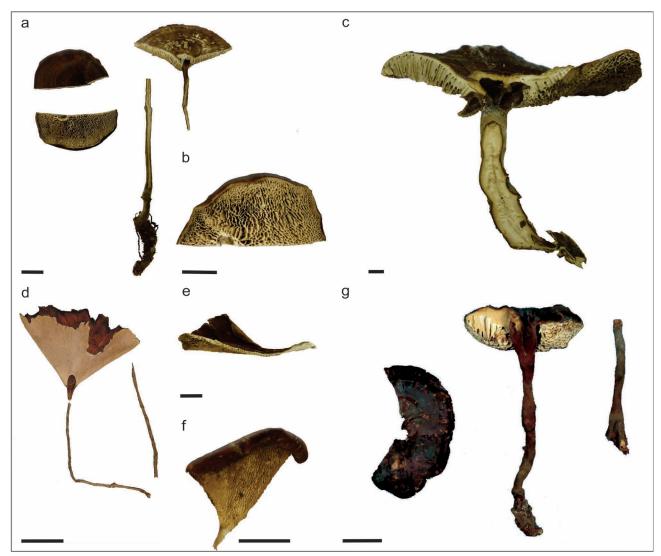


FIGURE 3. Macroscopic view of *A. calcitum* and related species. a—c *A. calcitum*: a—general view (FLOR52230, holotype); b—detail of hymenophore (FLOR52230); c—section of basidioma (FLOR 52232). d—f *A. partitum*: d—general view (K180681 type); e—detail of context and tubes (FLOR 48461); f—detail of hymenophore (FLOR 48461). g—general view of *Polyporus torrendi* = *A. calcigenum* (US 307270, holotype). Scale bar 1 cm. Photos by: D.H. Costa-Rezende

Basidiomata stipitate, mesopodal, pleuropodal or pseudomesopodal, single to occasionally two fused pilei, with superposed pileus (unusual) on the upper surface; *pileus* 25–70 mm in diameter, up to 6.0 mm thick, circular, subinfundibuliform 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 shiny, 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 yellow 2.5Y (8/4), with or without one or two brown lines coming from the stipe and a thin dark cuticle, 0.5–1 mm thick. *Tubes* concolorous with context, up to 5 mm long. *Pore surface* concolorous 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, 1–2 (3) per mm, (400) 450–900 (1000) μ m in diameter, (ave = 657.9 μ m); *dissepiment* entire or lacerate to almost irpicoid, (50) 60–180 (200) μ m, (ave = 127.1 μ 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* concolorous with pilear context, with two brown bands at least near the pileus; *cuticle* distinct brown.

Pilear surface composed of clamped generative hyphae, 3–5 μm in diameter, thin to slightly thick-walled and intermixed with solid skeletal hyphae, both parallel to the contextual hyphae. *Hyphal system* dimitic; *context* composed of clamped generative hyphae, (2) 3–5 μm in diameter, hyaline, thin-walled; and skeletal hyphae aciculiform or with two terminal branches, 5–6 μm in diameter, up to 900 μm without branches, hyaline to pale yellowish, straight or tortuous, thick-walled to almost solid; *trama* of tubes composed by clamped generative hyphae, 3–5 μm in diameter, hyaline, thin-walled; and skeletal hyphae aciculiform or with few apical branches (2–4) in variable size, 4.5–6 μm in diameter, up to 700 μm long (basal clamp not observed), some skeletal hyphae with a few lateral aborted branches, both in the main stalk and branches, variably dextrinoid. *Hyphal pegs* conical, 130×20 μm, variably present. *Basidia* clavate, $35–50 \times 15–30$ μm. *Basidiospores* broadly ellipsoid to ellipsoid, 11–14 (15) × (7) 8–10.5 (11) μm, (ave = 12.8×9.4 μm), Q = 1.24–1.61, (ave-Q = 1.37), pale yellowish, double-walled with the inner layer finely and regular ornamented, IKI-.

Substrate:—Growing always on the ground associated with roots of undetermined angiosperms.

Distribution:—So far known from the Cerrado, in Mato Grosso state, Brazil.

Other 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, Costa-Rezende DHCR031 (FLOR 52168); 07 January 2013, Costa-Rezende DHCR104 (FLOR 52226); Costa-Rezende DHCR 111 (FLOR 52228); Costa-Rezende DHCR 106 (FLOR 52229); Costa-Rezende DHCR107 (FLOR 50931); 12 January 2014, Pereira-Silva LPS007 (FLOR 52234); Pereira-Silva LPS009 (FLOR 52231); Pereira-Silva LPS015 (FLOR 52233); Pereira-Silva LPS016 (FLOR 52235); Pereira-Silva LPS019 (FLOR 52236); Pereira-Silva LPS069 (FLOR 52232); Pereira-Silva LPS081 (FLOR 52237); Sitio Véu da Noiva, 27 November 2012, Alves-Silva GAS071 (FLOR 52238); Alves-Silva GAS072 (FLOR 52239); Alves-Silva GAS074 (FLOR 52240); Primavera do Leste, near to Lagoa Vô Pedro Piana, 27 March 2013, Costa-Rezende DHCR 130 (FLOR 52243); APP Instituto Matogrossense do Algodão, near to Córrego Alminhas, 22 December 2013, Alves-Silva GAS549 (FLOR 52241).

Comments:—Amauroderma calcitum is macroscopically characterized by its robust basidiomata (pileus 2.5–6.0 mm thick) with a reddish brown, slightly shiny pilear surface, a pale context with or without brown bands, a brown, robust (in mature specimens) and slightly velvety stipe up to 5 mm in diameter, and large pores with lacerate dissepiments in mature specimens. Microscopically, it has a dimitic hyphal system with skeletal hyphae variably dextrinoid and broadly ellipsoid to ellipsoid, pale yellowish, double-walled basidiospores, with the inner layer fine and regularly ornamented. It could be compared with *A. calcigenum* (Fig. 3g) and *A. partitum* (Fig. 3d–f). *A. calcigenum* differs from *A. calcitum* by having a softer pileus and larger basidiospores [(12) 13.5–16 × 10–12 (12.5) μ m, (ave = 14.2 × 10.9 μ m)] (Table 3, Figs. 4, 5). *A. partitum* differs mainly by having thin and pliable basidiomata (up to 1.1 mm thick) and slightly smaller basidiospores [10–13 (13.5) × 8–10 (10.5) μ m (ave = 11.6 × 8.9 μ m)] (Table 3, Figs. 4, 5).

TABLE 3. Basidiospores size of Amauroderma calcitum and related taxa.

Taxa	Basidiospores (μm)	Ave. (μm)
A. calcitum		
FLOR 52230	11–13.5 × 8–10	12.4×9.25
FLOR 52228	11–14 × 9–10 (11)	12.6×9.5
FLOR 52238	11–14 × (8) 8.5–10 (10.5)	12.5×9.2
FLOR 52239	(12) 12.5–14.5 (15) × 9–10.5 (11)	13.4×9.85
SP 102719	12–14 × (8) 9–10	13.2×9.5
FLOR 52243	(11) 12–14 (15) × 8.5–10	12.8×9.25
FLOR 52234	(11) 11.5–13 (14) × (7) 8–10 (11)	12.2×8.9

TABLE 3. (Continued)

Taxa	Basidiospores (μm)	Ave. (μm)
FLOR 52232	11–14 × (8) 8.5–10	12.3 × 9.2
Average		12.8×9.4
A. partitum		
FLOR 48461	(10) 10.5–12 (13) × (7) 8–9.5 (10)	11.3×8.5
URM 82872	10–11.5 (12) × (7) 7.5–9	10.8×8.4
FLOR 44756	11–13 (13.5) × (8) 8.5–10	12 × 9.1
FLOR 45554	11–13 × (7.5) 8–10 (10.5)	12.3×8.9
Average		11.6 × 8.9
A. calcigenum		
URM 80725	(12) 13–15.5 × (10) 10.5–12	14.5 × 11.2
URM 80661	13–16 × 10–12	14.7 × 11.1
URM 80634	(13) 14–15.5 (16) × (10) 10.5–12	14.6 × 11.1
URM 82777	(11.5) 12–13.5 (14) × 10–11	12.3×10.3
FLOR 52315	12–15 (16) × 10–11 (11.5)	13.6×10.7
FLOR 52316	13–15 (16) × (9.5) 10–12	14.4×10.9
BPI 237146	(13) 13.5–16 × 10–12 (12.5)	14.8 × 11.1
BPI 302396	$(13.5)\ 14-16\ (16.5) \times 10-12\ (12.5)$	15 × 11.2
BPI 307270	(12) 13–15 × (9) 10–12 (12.5)	13.7×10.9
Average		14.2× 10.9

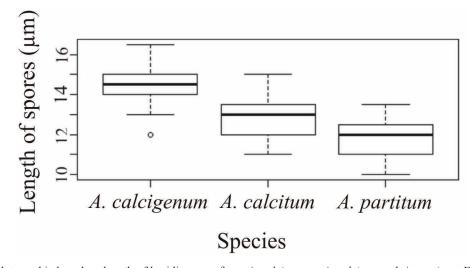


FIGURE 4. Boxplot graphic based on length of basidiospores from *A. calcigenum*, *A. calcitum* and *A. partitum*. For each taxon 25–75 percent quartiles are drawn using a box, median is shown with a horizontal line inside the box. The intervals represented by a line corresponding to the largest value less than 1.5 times the box height and the smallest value less than 1.5 times the box height. Values outside these intervals are considered outliers and are represented as circles.

Ryvarden (2004) describe the basidiospores of A. calcigenum with a slight difference in length and width from our measurements (12–15 x 9–12 µm). In relation to the spore measurements of A. partitum, our data agree with the the description given by Gomes-Silva et al (2010) [10–13 × 7–9.5(10) µm], but Ryvarden (2004) describes the spores as being a little longer [(10) 12–15 x 8–10]. The specimens studied by Corner (1983) and identified as A. calcigenum, collected at Mato Grosso and possibly Manaus (Amazon state), should be re-evaluated, since they have basidiospores similar in length and width to A. calcitum (12–14.5 x 8.5–10). These specimens also possess strongly dextrinoid skeletal hyphae. However, in Corner's description of a variety (collections from Rio de Janeiro and Manaus) with a minor difference in basidiospore measurement that he called A. calcigenum var. A, he suggested that the dextrinoidity of hyphae may not even be specific (Corner 1983).

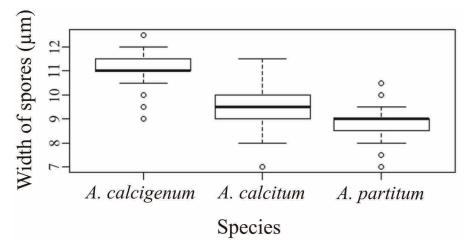


FIGURE 5. Boxplot graphic based on width of basidiospores from *A. calcigenum*, *A. calcitum* and *A. partitum*. For each taxon 25–75 percent quartiles are drawn using a box, median is shown with a horizontal line inside the box. The intervals represented by a line corresponding to the largest value less than 1.5 times the box height and the smallest value less than 1.5 times the box height. Values outside these intervals are considered outliers and are represented as circles.

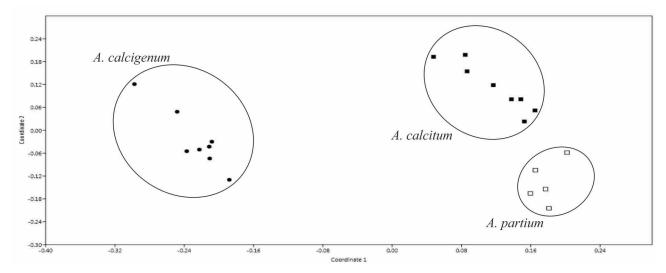


FIGURE 6. Non-metric multidimensional scaling (NMDS) plot of *A. calcigenum*, *A. calcitum* and *A. partitum*. Dots represents *A. calcigenum*, filled squares *A. calcitum* and non-filled squares *A. partitum*.

Notes on taxonomy and distribution of Amauroderma species (Ganodermataceae) from the Brazilian Cerrado

Amauroderma aurantiacum (Torrend) Gibertoni & Bernicchia, *Mycotaxon* 104: 322 (2008). Figs. 7 a−b, 8 a ≡ *Ganoderma aurantiacum* Torrend, *Iconographia mycologica* 21: 1007 (1932)

Description:—Furtado (1981) and Ryvarden (2004) as A. macrosporum J.S. Furtado (1981: 59).

Substrate:—Growing out of the ground associated with roots.

Distribution:—Brazil and Venezuela. In Brazil this species has been recorded from Amazonia, Atlantic Forest and the Cerrado (Ryvarden & Iturriaga 2001, Ryvarden 2004, as *A. macrosporum*, Gibertoni *et al.* 2008, Campacci & Gugliotta 2009, Gugliota *et al.* 2015).

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 15 May 2011, Costa-Rezende DHCR30 (FLOR 52167); 12 January 2014, Pereira-Silva LPS02 (FLOR 52197); Pereira-Silva LPS03 (FLOR 52203); Pereira-Silva LPS04 (FLOR 52202); Pereira-Silva LPS08 (FLOR 52199); Pereira-Silva LPS12 (FLOR 52200); Pereira-Silva LPS14 (FLOR 52201); Pereira-Silva LPS17 (FLOR 52204); Pereira-Silva LPS25 (FLOR 52198); Sitio Véu de Noiva, 19 January 2012, Alves-Silva LPS77 (FLOR 52206).



FIGURE 7. Macroscopic view of basidiomata of *Amauroderma* species. a, b *A. aurantiacum*: a—section of the basidioma (FLOR 52198); b—pilear surface in detail (FLOR 52198). c—general view of *A. brasiliense* (FLOR 50932). d, e—*A. omphalodes* (FLOR 52224). d—pilear surface in detail; e—section of basidioma. f,g—*A. praetervisum* (FLOR 52209). f—pilear surface in detail; g—section of basidioma. h—general view of *A. schomburgkii* (FLOR 52176). i—general view of *A. sprucei* (FLOR 52184). Scale bar: 1 cm. Photos by: D.H. Costa-Rezende.

Comments:—The thick and soft pileus when fresh, with orange to reddish brown pilear surface, large pores (1-2/mm) and globose to subglobose [(13) 14–17 (18) × 13–17 (18) μ m], yellow, and thick-walled spores, with conspicuous endosporic sub-reticulate ornamentation, similar to those described by Ryvarden (2004) and Furtado (1981), are distinctive for this species. Amauroderma calcigenum also presents a reddish brown pilear surface, soft pileus and big basidiospores, nonetheless the spores are ellipsoid with columnar ornamentation.

The presence of trichodermal structures in the pilear surface was not reported by Furtado (1981) and Ryvarden (2004), probably due to the specimens revised being too old, which may preclude the observation of this feature, as observed by us. However, we have observed trichodermal structures in well-preserved specimens, composed of loose generative hyphae, up to 120 μ m long, with or without clamps and adventitious septa. These generative hyphae are found projecting from a layer formed by moderately thick-walled generative hyphae, parallel to the context hyphae.

Amauroderma brasiliense (Singer) Ryvarden, *Syn. Fung.* 19: 44 (2004). Figs. 7 c, 8 b ≡ *Scutiger brasiliensis* Singer, *Beihefte zur Nova Hedwigia* 77: 22 (1983)

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

Substrate:—On the ground.

Distribution:—Brazil and Venezuela. In Brazil, it has been recorded from Amazonia and Atlantic Forest (Ryvarden 2004, Coelho *et al.* 2007, Campacci & Gugliotta 2009, Gugliotta *et al.* 2015), and is reported for the first time from the Cerrado.

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 50932).

Comments:—The soft basidiomata when fresh, a pale context dominated by clamped generative hyphae, the dimitic trama of the tubes with the presence of arboriform skeletal hyphae, as well the globose to ellipsoid spores [(6) $7-10 \times (5.5)$ 6–8 (9) µm] characterize this species.

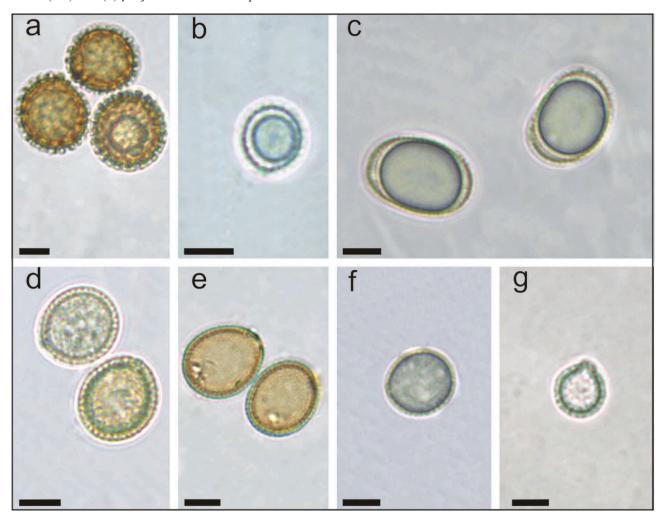


FIGURE 8. Spores of *Amauroderma* species from the Cerrado. a—*A. aurantiacum* (FLOR 52199). b—*A. brasiliense* (FLOR 50932). c—*A. calcitum* (FLOR52230, holotype) d—*A. omphalodes* (FLOR 52221). e—*A. praetervisum* (FLOR 52208). f—*A. schomburgkii* (FLOR 52176). g—*A. sprucei* (FLOR 52184). Scale bar: 5 μm. Photos by: D.H. Costa-Rezende.

The descriptions found in the literature indicated a heterogeneity to define the hyphal system and basidiospore shape of this species. Singer [1983, as *Scutiger brasilienses* Sing. (1983: 22)] considered the hyphal system as monomitic, while Ryvarden (2004) described it as dimitic, with skeletal hyphae scattered in the basidioma, some of them with a swollen apex (maybe clamydospores). On the other hand, Coelho *et al.* (2007) considered it monomitic to probably dimitic, with long and thick-walled contextual hyphae sometimes resembling skeletal hyphae, with basal clamps.

Gomes-Silva *et al.* (2015) pointed out that the species possesses rare skeletal hyphae, and wide, conspicuously clamped generative hyphae. The dendrohyphidia, gloeoporus hyphae and gloeocystidia mentioned by Coelho *et al.* (2007) were not observed by us, probably due to the fact that this kind of structure often collapses during drying.

Additionally, we consider that the context is composed only of clamped generative hyphae, thin to slightly thick-walled, some distinctly wider, with a swollen apex, up to 30 µm wide. Finally, we observed the dimitic hyphal system only in the trama of the tubes, composed of clamped generative and arboriform skeletal hyphae. Ryvarden (2004) probably observed the arboriform skeletal hyphae, as illustrated in his book.

Considering the basidiospores Ryvarden (2004) describes the shape as globose (7–9 μ m diam.), while Gomes-Silva *et al.* (2015) describe it as globose to subglobose ([8–10 (–11) × 7–9 μ m]. Only Coelho *et al* (2007) use the Q value and define the shape as subglobose to broadly-ellipsoid [(5.6–)6–9.2(–10) × (4.4–)5.2–7.2(–8) μ m] which is similar to our observations [Q = (1.0) 1.07–1.43(1.5), ave-Q = 1.21]. *Amauroderma trichodermatum* also has a monomitic context, but mainly differs by presenting a hairy pilear surface (Robledo *et al.* 2015).

Amauroderma omphalodes (Berk.) Torrend, Brotéria Série Botânica 18: 131 (1920). Figs. 7 d–e, 8 d
≡ Polyporus omphalodes Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 172 (1856a)

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

Substrate:—Growing out of soil but associated with roots.

Distribution:—Brazil to Belize. In Brazil, this species has been recorded from Amazonia, Atlantic Forest and Cerrado (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Gugliotta *et al.* 2015).

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Véu de Noiva, 19 February 2012, Alves-Silva GAS s/n (FLOR 52222); 19 February 2012, Alves-Silva GAS90 (FLOR 52218); 19 February 2012, Alves-Silva GAS131 (FLOR 52221); 20 May 2012, Alves-Silva GAS171 (FLOR 52217); 19 February 2012, Alves-Silva GAS172 (FLOR 52225); 19 February 2012, Alves-Silva GAS198 (FLOR 52223); 01 May 2013, Alves-Silva GAS475 (FLOR 52224); Sítio Vale do Rio Claro, 07 January 2013, Costa-Rezende DHCR112 (FLOR 52216).

Comments:—Amauroderma omphalodes is characterized by its stipitate, dull, very pale brown to dark reddish brown pileus, a pale context with dark bands from the stipe and globose to broadly ellipsoid spores, (10) 11-14 (15) × (8) 9-12 (13) μ m, that are pale yellow, thick-walled and have conspicuous ornamentation.

It shares similarities with *A. camerarium*, *A. praetervisum*, *A. pseudoboletus* (Spegazzini) J.S. Furtado (1968: 230) and *A. sprucei*. *Amauroderma camerarium* also has a pale context and large thick-walled spores with conspicuous ornamentation, however it differs in having a homogeneous context (Ryvarden 2004, Gomes-Silva *et al.* 2015). *Amauroderma praetervisum* and *A. pseudoboletus* also have black bands in the context and large spores; however, the paler, thinner walls and less conspicuous endosporic ornamentation of spores from *A. praetervisum* are distinctive features. Furthermore, *A. praetervisum* has a darker pilear surface. *Amauroderma pseudoboletus* has larger pores, 3-5/mm [(210) 250–350 (400) vs (3) 4–7/mm, (80) 110–210 (230) μ m in diameter]. *Amauroderma sprucei* also has a pale context and small pores, and can be distinguished mainly by its pale ochraceus to orange pore surface (usually observed in many specimens) and the smaller spores [(7) 8–10 × 7–9 vs (10) 11–14 (15) × (8) 9–12 (13) μ m]. According to the literature, the basidiospores of *A. omphalodes* are described as globose to subglobose (Furtado 1981, Ryvarden 2004, Gomes-Silva *et al.* 2015), which differs from our observations of globose to broadly ellipsoid spores (Q = 1.00–1.30, ave-Q = 1.16).

Amauroderma praetervisum (Pat.) Torrend, Brotéria, sér. bot. 18: 131 (1920). Figs. 7 f−g, 8 e ≡ *Ganoderma praetervisum* Pat., *Bulletin de la Société Mycologique de France* 5: 78 (1889)

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

Substrate:—Out of soil but associated with roots.

Distribution:—Brazil to Mexico. In Brazil this species has been recorded from Amazonia, Atlantic Forest, Caatinga and Cerrado (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Abrahão *et al.* 2012, Gugliotta *et al.* 2015).

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 12 January 2014, Pereira-Silva LPS013 (FLOR 55263); 12 January 2014, Pereira-Silva LPS066 (FLOR 52209); 12 January 2014, Pereira-Silva LPS067 (FLOR 52208); 12 January 2014, Pereira-Silva LPS068 (FLOR 52207).

Comments:—The pale context with two black bands and a thin dark cuticle, small pores (4–5/mm) and a distinct core in the stipe, which is paler and softer than the outside layer, characterize the species macroscopically. Microscopically, it is recognized by the combination of a crust composed of some free hyphae on the pilear surface, trama of tubes having variable dextrinoid skeletal hyphae and large, subglobose to broadly ellipsoid spores, (11.5)

 $12-13.5(14) \times 10-12(13) \mu m$.

Regarding the pale context, consistency of pileus and pore size, A. omphalodes and A. sprucei could be morphologically related. Amauroderma omphalodes was treated above and A. sprucei has a whitish context and smaller basidiospores [(7.0) 8.0–10 × 7–9 µm], as well as an orange pore surface in many specimens. Amauroderma schomburgkii also has a robust pileus, with similar consistency, black bands in the context and small pores, but differs in its darker context, smaller spores and a cortex in the pilear surface. Amauroderma pseudoboletus presents a context with similar color and similar spore size, and can be distinguished by its conspicuous ornamentation of spores. Furtado 1981, Ryvarden 2004 and Gomes-Silva et al. 2015 described the basidiospores as globose to subglobose, differing from our observations of subglobose to broadly ellipsoid [$Q = (1.07) \cdot 1.08-1.27 \cdot (1.30)$, ave-Q = 1.18].

Amauroderma schomburgkii (Mont. & Berk.) Torrend, Brotéria, sér. bot. 18: 140 (1920). Figs. 7 h, 8 f ≡ *Polyporus schomburgkii* Mont. & Berk., *London Journal of Botany* 3: 331 (1844)

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

Substrate:—Growing out of soil but associated with roots.

Distribution:—The species seems to be the most common *Amauroderma* in the neotropics, occurring from South Brazil to Central America. In Brazil, it has been recorded from the Amazon, Atlantic Forest and Cerrado (Furtado 1981, Ryvarden 2004, Campacci & Gugliotta 2009, Gugliotta *et al.* 2015).

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Véu da Noiva, 10 March 2013, *Alves-Silva* GAS393 (FLOR 52176); 10 March 2013, *Alves-Silva* GAS401 (FLOR 52175)

Comments:—This species is recognized by its brown context with dark bands, pileipellis as a cortex, small pores (5-7/mm) and globose to sub-globose (rarely broadly ellipsoid) basidiospores $[(8) 9-12 \times (7) 9-11 \,\mu\text{m}]$. Amauroderma exile and A. sprucei also have small pores and similarly shaped basidiospores. However, A. exile has a shiny, reddish brown and flexible pileus, distinct from A. schomburgkii, which usually presents a dull and hard basidiomata. Amauroderma sprucei is easily distinguished by its whitish context, vivid orange pore surface in some specimens, dextrinoid skeletal hyphae and a pileipellis as a crust.

Corner (1983) studied some specimens that he called *A. schomburgkii* A and *A. schomburgkii* B, with a distinct distribution in Brazil (the A in Amazonia, the B in Atlantic rain forest), but both had subglobose basidiospores (9–11.5 x 8–10.5 μ m), different to the information given by Furtado (1981), Ryvarden (2004) and Gomes-Silva *et al.* (2015), in which the shape is described as globose with a variation in the diameter (between 7–11 μ m, in general). As we have obtained the Q = 1.00–1.12 (1.20), ave-Q = 1.09, we describe the shape as globose to subglobose (rarely broadly ellipsoid).

Amauroderma sprucei (Pat.) Torrend, Brotéria, sér. bot. 18: 121 (1920). Figs. 7 i, 8 g

- ≡ Porotheleum rugosum Berk., Hooker's Journal of Botany and Kew Garden Miscellany 8: 237 (1856b)
- ≡ Ganoderma sprucei Pat., Bulletin de la Société Mycologique de France 10 (2): 75 (1894)

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

Substrate:—On the ground, associated to roots.

Distribution:—Brazil, Venezuela, French Guyana, Costa Rica, Belize and Cuba. In Brazil this species has been recorded from Amazonia, Atlantic Forest and Caatinga (Furtado 1981, Decock & Herrera-Figueroa 2006, Campacci & Gugliotta 2009, Drechsler-Santos *et al.* 2013, Gugliotta *et al.* 2015). Corner (1983) presented the species (as *A. dubiopansum*) from the north of Nova Xavantina, Mato Grosso, which is a transition between the Cerrado and Amazonian vegetation.

Specimens examined:—BRAZIL. Mato Grosso: Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, Sítio Vale do Rio Claro, 07 January 2013, Costa-Rezende DHCR113 (FLOR 52191); 07 January 2013, Costa-Rezende DHCR114 (FLOR 52184); 07 January 2013, Costa-Rezende DHCR115 (FLOR 52192); 12 January 2014, Pereira-Silva LPS21 (FLOR 52190); 12 January 2014, Pereira-Silva LPS22 (FLOR 52189); 12 January 2014, Pereira-Silva LPS78 (FLOR 52186); 12 January 2014, Pereira-Silva LPS79 (FLOR 52185).

Comments:—The white context, a vivid orange pore surface in some specimens, small pores [5–7 (8) pores/mm], pilepellis as a crust with some free hyphal tips, the strongly dextrinoid skeletal hyphae and predominantly subglobose spores [(7.0) $8.0-10 \times 7-9 \mu m$] with conspicuous columnar ornamentation, are typical for this species. Regarding the

pale context, consistency of pileus and pore size, A. omphalodes and A. praetervisum are morphologically related, but distinguished by presenting larger spores [(11.5) 12–13.5 (14) × 10–12 (13) and (10) 11–14 (15) × (8) 9–12 (13), respectively] than A. sprucei. Furtado (1981) and Ryvarden (2004) described the shape of the basidiospores as globose, which differs from the observation of Corner (1983, as A. dubiopansum), who considered the basidiospores as globose to subglobose. According to our observations, the basidiospores are globose to subglobose (Q = 1.0-1.15, ave-Q = 1.13). The range from ochraceus to orange in the hymenophore coloration was also observed by Decock & Herrera-Figueroa (2006), who synonymized A. dubiopansum and A. sprucei mainly based in the plasticity of this feature.

Annotated key to Amauroderma species that occur in Brazil

1	Hyphal system monomitic or mostly so	
2	Pilear surface glabrous Pilear surface strigose	
3 -	Hymenophore with large pores, up to 4 /mm	
4 -	Pilear surface laccate	
5	Basidiospores globose to subglobose Basidiospores ellipsoid	
6	Basidiospores with a semi-reticulate ornamentation pattern, 13–17 (18) µm wide width	
7	Pilear surface composed of swollen ventricose apical structures Pilear surface trichodermal	
8	Pileus thin and pliable, up to 2 mm thick	
9	Pileus coriaceous, basidiospores up to $11-14$ (15) × (7) $8-10.5$ (11) μm (average size 12.8×9.4) Pileus soft, basidiospores larger (12) $13.5-16 \times 10-12$ (12.5) μm (average size 14.1×10.9)	
10	Basidiospores with inner layer smooth	
11 -	Basidiomata sessile to sub-, laterally stipitate Basidiomata distinctly stipitate	
12.	Basidioma sessile, abhymenial surface azonate, black at the base, pores angular, dissepiment lacerate subglobose, 11–14 × 10–13 μm	
13	Stipe laccate	
14	Pores 7–8/mm Pores 5–6/mm	-
15 -	Stipe whitish	
16 -	Basidiospores oblong to obovoid	
17 -	Basidiospores oblong	
18	Basidiospores up to 11 µm wide	19

-	Basidiospores wider	23
19 -	Basidiomata with 2–9 lobes	
20	Basidiospores distinctly ellipsoid Basidiospores globose to sub-globose	
21	Context whitish to pale yellow, pore surface almost white to orange, pilear surface like a trichoderm	
22	Basidiomata usually with a shiny reddish brown pilear surface and flexible pileus	
23	Pileus up to 1 cm in diameter, basidiospores 15–17 \times 13–15 μm Pileus more than 1 cm in diameter, basidiospores up to 15 \times 13 μm	
24	Basidiospores thin to slightly thick-walled, finely ornamented	
25 -	Pileus often soft to corky, hymenophore with 3–5 pores/mm	
26	Context without dark bands Context with two dark bands	

Discussion

In our study, *Amauroderma*, in its current morphological sense (Furtado 1981, Ryvarden 2004), is polyphyletic both in nrITS and combined analyses (nrITS + nrLSU). The nrITS dataset is more inclusive, while the nrITS + nrLSU dataset represents a narrower sampling. However, three distinct clades were recovered in both analyses.

Gomes-Silva *et al.* (2015), presenting the first phylogeny of this genus also observed a similar topology, and considered *Amauroderma* as a non-monophyletic entity. They also speculated that the clade that contains most of the Brazilian species could be considered *Amauroderma sensu stricto*, and the remaining *Amauroderma* clades could represent new genera. The same authors also presented a topology obtained from LSU analyses with three neotropical *Amauroderma* species, which clustered in a moderated to well supported clade (83 MP, 80 ML, 1.00 PP).

Considering the nrITS analyses, clade A contains only neotropical species, and comprises the specimens of the type species of the genus, *A. schomburgkii* (=Fomes regulicolor). Since this clade is significantly supported in our phylogenetic analyses (1.00 BPP, 70% BS), being morphological and geographically homogeneous, and contains specimens of the type, we also consider this group as *Amauroderma sensu stricto*, also with the inclusion of three species (*A. calcitum*, *A. intermedium* and *A. pseudoboletus*), which were not available in the previous phylogenetic analyses (Gomes-Silva *et al.*, 2015). The species in this clade share a sessile to stipitate basidiomata with a di-trimitic hyphal system, composed of clamped generative hyphae, arboriform to skeleto-binding hyphae (both in context and tubes) and non-truncated, double-walled spores with columnar to semi-reticulate endosporic ornamentation. A similar clade, however, with *A. brasiliense* in a long branch, was recovered by the combined analyses without statistical support (clade A) (Fig. 2).

Amauroderma sprucei grouped as a sister clade (0.99 BPP, 56% BS) to *G. coffeatum* J.S. Furtado (1967: 383) in an internal branch of a more inclusive clade composed mostly of dull and laccate *Ganoderma* species in the ITS analyses (clade B, Fig. 1). In the combined analyses, it grouped with sequences of the genus *Tomophagus* without statistical support (clade B, Fig. 2). *Amauroderma sprucei* is a common neotropical species and does not share morphological features with either *Ganoderma* or *Tomophagus*, which could explain the conflicting topologies. The species is characterized by a whitish context, an ochraceus to vivid orange pore surface in some specimens, strongly dextrinoid skeletal hyphae and predominantly subglobose spores [(7.0) $8.0-10 \times 7-9 \mu m$], with conspicuous columnar ornamentation (Decock & Herrera-Figueroa 2006). Thus, further morphological and phylogenetic studies should be performed to clarify its relationships at generic level.

The remaining *Amauroderma* species are grouped in clade C in the ITS analyses as a sister clade of *Tomophagus*, without statistical support. Four species form this clade: *A. brasiliense*, *A. rude* (Berk.) Torrend (1920: 127), *A.*

rugosum (Blume & T. Nees) Torrend (1920: 127) and A. subresinosum (Murrill) Corner (1983: 93). Gomes-Silva et al. (2015) also showed a topology in which these species did not group with the putative Amauroderma s.s. senso authors. Amauroderma brasiliense is a distinctive neotropical species due to its monomitic context (see remarks in taxonomy). This feature (differing from the species in Amauroderma s.s.), combined with the molecular evidence showed by the topologies of distinct analyses (our work and Gomes-Silva et al. 2015), is good evidence that future studies may suggest this taxon is a new genus in which A. trichodermatum should be likely included, as speculated by Robledo et al. (2015), since these are the only two species that share the monomitism in the family.

Amauroderma rude [in the sense of A. rude var rude of Furtado (1981)] occurs in Africa, Asia and Oceania, and is mainly characterized by a soft, corky to spongy basidiomata, trichodermal pilear surface and globose to subglobose, $9-11 \times 7.5-9 \mu m$, double-walled basidiospores with an ornamented inner layer (Furtado 1981). Amauroderma rude (Berk.) Torrend var intermedium J. Furtado (1968: 251) was proposed by Furtado (1968) based on morphological similarities among Amauroderma rude and neotropical species under different names, such as A. intermedium (Bres. & Pat.) Torrend (1920: 128) and its synonyms. Although the Index Fungorum (www.indexfungorum.org) database includes A. intermedium as a synonym of A. rude, according to our topology, A. intermedium and A. rude are distantly related, justifying the separation between A. rude and A. intermedium at species level, as already observed by Ryvarden (2004) and Gomes-Silva et al. (2015), based on morphological analyses. Index Fungorum (www.indexfungorum.org) also includes A. pseudoboletus as a synonym of A. rude, which is refuted by our topology (Fig. 1). Though A. rude and A. pseudoboletus might present similar soft basidiomata, the former presents smaller basidiospores [9–11 × 7.5–9 μ m vs 12–14 × (10) 11–13 μ m]. In addition, A. pseudoboletus is restricted to the Neotropics, while A. rude occurs in Africa, Asia and Oceania (Furtado 1981).

Within clade A, the new species A. calcitum is corroborated by phylogenetic analyses, with all specimens grouping in a well-supported terminal clade (1.0 BPP, 100% BS), appearing as a sister group (1.0 BPP, 98% BS) of A. partitum (Fig. 1), as already expected due to its similar morphology (discussed below). Additionally, it is distantly related from the other similar species, A. calcigenum. The specimens of this species grouped in two distinct, unrelated terminal branches, one of them representing a Brazilian specimen while the other is from Argentina. Further studies are needed to improve the species delimitation in both cases.

Amauroderma calcitum is morphologically closely related to A. calcigenum (Fig. 3 g) and A. partitum (Fig. 3 d-f), mainly due to the color of the pilear surface and the 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, and the former author also included other taxa into the synonymy [Stereum miquelianum Mont (1851: 203), Hexagonia gracilis Berk. (1856b: 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 distinguish this taxon. Gomes-Silva et al. (2010), agreeing with Ryvarden (2004), observed that A. calcigenum has basidiomata with a thick pileus (up to 12 mm), while A. partitum has smaller and fragile basidiomata, with thinner stipe and pileus, usually pliable. Microscopically, A. calcigenum has slightly longer and wider basidiospores. Gomes-Silva et al. (2015) corroborated the distinction between the two taxa with phylogenetic analyses. In the same study, the authors pointed out that A. partitum is a macromorphological variable species, presenting specimens with small pileus, with cream, orange to pale brown abhymenial surface, large pores (1/mm) and lacerate dissepiments; and specimens with larger pileus, with pale brown to brown abhymenial surface, smaller pores (3/mm) and entire dissepiments. Despite the fact that measurements of macroscopic features such as pileus size and thickness were not presented by Gomes-Silva et al. (2015), it is possible to observe in their plate (Fig. 9: 18) that even in the group with larger pileus, it is thin and pliable, as pointed by Gomes-Silva et al. (2010).

Regarding the pilear thickness and basidiospore size, A. calcitum shows an intermediate morphology between A. calcigenum and A. partitum, i.e., the robust basidiomata are similar to those of A. calcigenum and the size and shape of spores are similar to those of A. partitum (Table 3, Figs. 4, 5). The overlap or intermediate features suggest that these taxa could be interpreted as synonyms; however, the detailed morphological study of the specimens, including type collections and reference materials, led us to conclude that there are three distinct species. Amauroderma calcitum has a thicker basidiomata than A. partitum, both for pileus (up to 6 mm vs 1.1 mm thick) and stipe (up to 5 mm vs 1,5 mm thick), larger pores $[(400) \ 450-910 \ (1000) \ vs \ (300) \ 350-650 \ (1000) \ \mu m$ in diameter] and larger basidiospores $[11-14 \ (15) \times (7) \ 8-10.5 \ (11) \ \mu m$, (ave = $12.8 \times 9.4 \ \mu m$) $vs \ [10-13 \ (13.5) \times 8-10 \ (10.5) \ \mu m$ (ave = $11.6 \times 8.9 \ \mu m$)]. Some primordial specimens of A. calcitum seem similar to A. partitum but these specimens are not fertile yet, with clearly non-developed tubes and pores. The specimen SP 102719, considered by Furtado (1981) as A. calcigenum, is treated here as A. calcitum. It is possible that specimens with intermediate morphology led the author to synonymize A. calcigenum and A. partitum; a similar criterion was used by him to synonymize Fomes paulensis Hennings (1904: 202)

in *A. sprucei*. 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*, since both species have thick pilei. On the other hand, *A. calcigenum* presents a softer pileus, darker context and larger basidiospores than *A. calcitum* [(12) $13.5-16 \times 10-12$ (12.5), (ave = $14.2 \times 10.9 \, \mu m$)]. Type specimens of *A. brockesii* and *A. torrendii* (synonyms of *A. calcigenum*) also present larger basidiospores, with a similar range as *A. calcigenum*.

The NMDS analyses (Fig. 6) show three distinct groups, reinforcing the distinction between the three taxa, based on basidiospore size, pore diameter, dissepiment thickness, pores per millimeter, pilear surface microstructures, pileus consistency and thickness. Despite the overlap in the basidiospores size range, the boxplots both for basidiospores length and width show that each of the three species exhibits a particular distribution of the data, regarding mathematic measurements like quartiles and medians (Figs. 4, 5).

The specimen called A. aff. praetervisum presents smaller spores than A. praetervisum ([9.5] 10-11 [12] \times 9-11 µm vs [11.5] 12-13.5 [14] \times 10-12 [13] µm) and clustered in a well-supported clade (1.0 BPP, 99% BS) with one specimen identified as A. praetervisum which might be conspecific. Since there is a second clade made up of three A. praetervisum specimens (JX310856, KC348460, JX310855) further studies with detailed morphological comparison might reveal a new species. The sample called Amauroderma sp. (KR816525) is an immature Amauroderma specimen that was collected in the Brazilian Amazonian and could not be delimited neither with morphology or molecular phylogeny.

In relation to the large macro and micromorphological similarities between *A. omphalodes* and *A. camerarium*, it is important to highlight that the type specimens of *A. camerarium* (*Polyporus camerarius*, Spruce 171 and 197) and of *A. omphalodes* (*Polyporus omphalodes*, Spruce 32 and 194) were all collected in the locality of Panuré (Brazilian Amazon), and both species were described by Berkeley (1856a), who did not make a morphological comparison between these taxa. Moreover, it is not possible to detect a substantial difference between them when the protologues are compared. Furtado (1981), in a taxonomic review of *Amauroderma*, pointed out that the distinction of those species is based on the microstructures of the pilear surface, *i.e.*, *A. camerarium* presents a cortex, while *A. omphalodes*, a crust; the cottony consistency of *A. omphalodes* when torn was recorded by the author as well. On the other hand, Ryvarden (2004) distinguished them by the dextrinoid skeletal hyphae in *A. camerarium*, and by the presence of one or two black lines in the context for *A. omphalodes*. However, the author mentioned a pilear surface made up of agglutinated hyphae, without any distinct structure for both species. Gomes-Silva *et al.* (2015) noticed the presence of lines in the context of *A. omphalodes*. It is important to note that Furtado (1981) did not study either Melzer's reaction or observe the presence of lines in the context.

The type specimen of *A. omphalodes* (Spruce 32) is well preserved and a pileipellis as a crust, composed by strongly thickened generative hyphae was easily observed, as well as two distinct dark lines in the context. In contrast, we analyzed only a small piece of the type of *A. camerarium* (Spruce 171) and the presence of lines, microstructures of the pilear surface and spores were not possible to observe. The specimen SP 61541, determined by Furtado (1981) as *A. camerarium*, also has lines in the context and a pileipellis as a cortex. Among our collections, we observed specimens with a pileipellis as a cortex (such as in SP 61541), as an indeterminate derm and as a young crust, the latter made up of slightly thick-walled, clamped generative hyphae, apparently without the wall deposition that Furtado (1981) called incrustations. In addition, dextrinoid reaction is variable among the studied collections.

Following the sense of Furtado (1981), some of our specimens could be identified as *A. camerarium* (the ones with a cortex) or as *A. omphalodes* (the ones with an undetermined derm or a crust). Since the structure in pilear surface seems to be quite variable, we identified our specimens as *A. omphalodes* due to the presence of lines in the context, following Ryvarden (2004) and Gomes-Silva *et al.* (2015). Further examination of other reference material and phylogenetic analyses may clarify the status of both taxa.

Since *Amauroderma* is a heterogeneous genus in its current sense, and our results as well as previous studies indicates that the genus is polyphyletic, further detailed morphological and phylogenetic studies, with inclusion of other Ganodermataceae sequences, including extra neotropical taxa, as well as other molecular markers, may clarify the relationships of *Amauroderma* species and the proposition of scientific novelties.

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