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Amauroderma (Ganodermataceae, Polyporales) – bioactive compounds, beneficial properties and two new records from Laos

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Abstract

Species of Ganodermataceae have been widely used as traditional medicines in Asia over many centuries. *Ganoderma* and *Amauroderma* are widely researched, owing to their beneficial medicinal properties. We surveyed species of *Amauroderma* in the Greater Mekong Subregion countries; China, Laos, Myanmar, Thailand and Vietnam. In this paper, we introduce two new records of *Amauroderma* from Laos; *Amauroderma pressuii* based on morphology and *A. rugosum* based on both morphology and molecular phylogenetic evidence. The collected species are described with coloured photographs and illustrations and compared with similar taxa. We also provide a phylogeny for *Amauroderma* based on ITS and LSU sequence data and the taxonomic status of the species is briefly discussed. In addition, we reviewed the bioactive compounds and beneficial properties of *Amauroderma*.

Key words – Medicinal properties – Morphology – Phylogeny – Two new records

Introduction

Ganodermataceae is a large family of Polypores with seven accepted genera: *Amauroderma*, *Foraminispora*, *Furtadoa*, *Ganoderma*, *Haddowia*, *Humphreya* and *Polyporopsis* (Richter et al. 2015, Costa-Rezende et al. 2017). The genus *Amauroderma* Murrill was described in 1905 based on *Fomes regulicolor* Cooke (1886) and typified by *Amauroderma regulicol*or (Berk. ex Cooke) Murrill (= *A. schomburgkii*) from Cuba (Torrend 1920). Torrend (1920) worked on the genus in South America based mainly on spore shape (globose or oblong, never truncate) and the presence of a stipe (usually dull, like the pilear surface) and published 28 species of *Amauroderma* placed within three sections. *Amauroderma* was carefully revised by Furtado (1981) and recognized 27 species. This genus has a tropical and subtropical distribution with the main centre of diversity in the Neotropics (Ryvarden 2004). *Amauroderma* species are usually found in associated with dead wood or roots of living or dead trees typically emerging from the ground and the mycelial phase is connected to the roots of living or dead trees which causes white rot (Furtado 1981, Ryvarden

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2004). There are 135 epithets listed in Index Fungorum (2018). The members of *Amauroderma* varies from stipitate to sessile basidiomata with a variably laccate or dull pileus, a trimitic hyphal system and ellipsoid, subglobose to globose bitunicate basidiospores with a smooth, semi-reticulate, honeycomb or asperulate to verrucose inner wall (Furtado 1981, Ryvarden 2004, Gomes-Silva et al. 2015, Li & Yuan 2015). Macroscopically, *Amauroderma* shares similarities with *Ganoderma* P. Karst. in the similar basidiocarp shape of the central or lateral stipe and laccate or dull surface. *Ganoderma* can be separated from *Amauroderma* by its distinctly truncate basidiospores, and most *Ganoderma* species grow on dead wood, while most *Amauroderma* species grow in the ground from buried roots/woods (Ryvarden 2004, Hapuarachchi et al. 2015). It was shown that *Amauroderma* is a non-monophyletic taxon based on comprehensive morphological and phylogenetic analyses by some researchers in Brazil (Gomes-Silva et al. 2015, Costa-Rezende et al. 2016).

Amauroderma species are regarded as economically valuable because of their important medicinal properties and pathogenicity (Dai et al. 2007, 2009, Jiao et al. 2013, Chan et al. 2013). Taxonomic studies of *Amauroderma* in Asia have been carried out over many years by various researchers (Teng 1936, Zhao et al. 1979, Zhao & Zhang 1987, Li & Yuan 2015, Song et al. 2016). Twenty species have been recorded in China (Zhao & Zhang 2000), but among these, only six have been confirmed as *Amauroderma* based on both morphological characters and phylogenetic analyses: *A. austrosinense* J.D. Zhao & L.W. Hsu, *A. concentricum* Song, Xiao L. He & B.K. Cui, *A. perplexum* Corner, *A. rugosum* (Blume & T. Nees) Torrend, *A. subresinosum* (Murrill) Corner and *A. yunnanense* J.D. Zhao & X.Q. Zhang (Li & Yuan 2015), while the other *Amauroderma* species recorded from China have not been fully studied yet. Furthermore, members of this genus have been subsequently recorded from the Greater Mekong Subregion countries such as Thailand (Chandrasrikul et al. 2011), Myanmar (Thaung 2007) and Vietnam (Quang et al. 2011). Here, we report on *A. pressuii* and *A. rugosum* as new records from Laos based on morphology and both morphology and molecular data, respectively. We provide a phylogeny for the *Amauroderma* based on combined ITS and LSU analyses. Moreover, we present and discuss experimental evidences in connection with *Amauroderma* and its beneficial medicinal properties.

Materials & Methods

Samples of *Amauroderma* were collected on July 2016 and June 2017 from Laos and dealt with as in Cao et al. (2012). The materials were deposited at Guizhou University (GACP) and Mae Fah Luang University (MFLU) herbaria.

Morphological characteristics examination

Macro-morphological characteristics were described based on fresh material, and on the photographs provided here. Colour codes (e.g. 5B5) are from Kornerup & Wanscher (1978). Specimens were dried and placed separately in plastic bags. For micro-morphological observations, basidiomes were examined under a stereo dissecting microscope (Motic SMZ 168 series) and sections were cut with a razor blade, mounted in 5% KOH, and then observed, measured and illustrated under a compound microscope (Nikon ECLIPSE 80i) equipped with a camera (Canon 600D). Measurements were made using Tarosoft (R) Image Frame Work v. 0.9.7. At least 20 basidiospores were measured from each mature specimen except for very scanty materials. The basidiospore size was measured both with and without the myxosporium, but only spore sizes with myxosporium were used for comparisons. Basidiospore dimensions are given as (a–) b–c–d (–e), where a represents the minimum, b (mean average-standard deviation), c the average, d (mean average+standard deviation) and e the maximum. *Q*, the length/width ratio (L/W) of a spore in side view and *Qm* is the average, smallest and largest *Q* values given as *Q*. Pellis sections were taken from the mature pileus portion and mounted in Melzer's reagent for observation. The Facesoffungi number is provided as explained in Jayasiri et al. (2015).

DNA extraction, PCR and sequencing

Dried samples of basidiomes were used to extract genomic DNA. Genomic DNA was extracted using an EZgene TM Fungal gDNA Kit (Biomiga, CA, USA) according to the manufacturer instructions. DNA concentrations were estimated visually in agarose gel by comparing band intensity with a DNA ladder 1Kb (Invitrogen Biotech). Reaction mixtures (50 µl) contained 2 µl template DNA (ca. 10 ng), 19 µl distilled water, and 2 µl (10 µM) of each primer and 25 µl 2x BenchTopTM Taq Master Mix (Biomigas). Amplification conditions were 40 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min for all DNA fragments. The ITS rDNA regions were amplified using the universal primer pair ITS4 and ITS5 (White et al. 1990). Amplified PCR products were verified by 1% agarose gel electrophoresis stained with ethidium bromide in 1x TBE. The PCR products were sequenced by SinoGenoMax Co., Ltd (Beijing).

Sequence alignment and phylogenetic analysis

The taxa information and GenBank accession numbers used in the molecular phylogenetic analyses are listed in Table 1. The quality of the newly obtained sequences from the *Amauroderma* specimens was checked by observing the chromatogram with BioEdit (Hall 1999) and by examining BLAST search results according to Nilsson et al. (2012). The BLAST search was also used to retrieve sequences from the closest matching taxa in *Amauroderma*. Forty-two nucleotide sequences representing 22 species of *Amauroderma* from Asia, Australia and South America were retrieved from GenBank. Those sequences and five newly generated sequences were aligned using MAFFT v. 7.309 (Katoh & Standley 2013) online at http://mafft.cbrc.jp/alignment/server/index.html, and the alignment was improved manually where necessary using Bioedit. Maximum likelihood (ML) analysis was performed using RAxML-HPC2 (Stamatakis 2014) on the CIPRES Science Gateway V. 3.3 (Miller & Blair 2009), with default settings except the number of bootstrap replicates was set to 1,000. For Bayesian (BY) analysis, GTR+I+G model of evolution was selected with MrModeltest 2.2 (Nylander 2004) as the best-fit model. BY analyses were conducted with two runs of six simultaneous Markov chains and trees were sampled every 100th generation. The analyses were stopped after 5,000,000 generations when the average standard deviation of split frequencies was below 0.01. The convergence of the runs was checked using TRACER v1.6 (Rambaut et al. 2013). The first 25% of the resulting trees were discarded as burn-in, and PP were calculated from the remaining sampled trees. In both ML and BY analyses, *Tomophagus colossus* was selected as the outgroup taxon. ML bootstrap values and Bayesian posterior probabilities greater than or equal to 70% and 0.95, respectively, were considered as significant support. The phylogenetic tree was visualized with FigTree version 1.4.0 (Rambaut 2012) available at http://tree.bio.ed.ac.uk/software/figtree/.

Table 1 Sequences used in the phylogenetic analysis

Species	Voucher/ Strain	Origin	ITS	LSU	References
Amauroderma aurantiacum	FLOR 52205	Brazil	KR816510	KU315205	Costa-Rezende et al. 2016
A. aurantiacum	URM 78847	Brazil	JX310840	JX310840	Gomes-Silva et al. 2010
A. austrosinense	Cui 13618	China	KU219973	KU219996	Song et al. 2016
A. calcigenum	URM 83864	Brazil	JX982565	JX982565	Gomes-Silva et al. 2010
A. calcigenum	URM 86847	Brazil	KT006601	KT006601	Gomes-Silva et al. 2010
A. calcitum	FLOR 50931	Brazil	KR816528	KR816528	Costa-Rezende et al. 2016

Table 1 Continued.

Species	Voucher/ Strain	Origin	ITS	LSU	References
A. calcitum	FLOR 52230	Brazil	KR816529	-	Costa-Rezende et al. 2016
A. camerarium	FLOR 52169	Brazil	KR816523	KR816523	Costa-Rezende et al. 2016
A. concentricum	Cui 12644	Sichuan, China	KU219974	KU219997	Song et al. 2016
A. concentricum	Cui 12648	Sichuan, China	KU219975	KU219998	Song et al. 2016
A. elegantissimum	URM 82787	Brazil	JX310843	KT006616	Gomes-Silva et al. 2010
A. elegantissimum	URM 82789	Brazil	JX310844	KT006617	Gomes-Silva et al. 2010
A. exile	URM 82794	Brazil	JX310845	JX310845	Gomes-Silva et al. 2010
A. floriformum	URM 83250	Brazil	JX310846	JX310846	Gomes-Silva et al. 2010
A. intermedium	FLOR 52246	Brazil	KR816524	KU315208	Costa-Rezende et al. 2016
A. intermedium	FLOR 52248	Brazil	KR816527	KU315209	Costa-Rezende et al. 2016
A. laccatostipitatum	HFSL (ACGS7)	Brazil	KT006602	-	Gomes-Silva et al. 2010
A. laccatostipitatum	URM 83238	Brazil	JX310847	JX310847	Gomes-Silva et al. 2010
A. omphalodes	MG (AS592)	Brazil	KT006603	-	Gomes-Silva et al. 2010
A. omphalodes	URM 84236	Brazil	KT006604	-	Gomes-Silva et al. 2010
A. partitum	URM 82884	Brazil	JX310851	-	Gomes-Silva et al. 2010
A. partitum	URM 83039	Brazil	JX310852	-	Gomes-Silva et al. 2010
A. perplexum	Cui 6496	Hainan, China	KJ531650	KU220001	Li & Yuan 2015
A. perplexum	Dai 10811	Hainan, China	KJ531651	KU220002	Li & Yuan 2015
A. praetervisum	REC 18707	Brazil	JX310855	-	Gomes-Silva et al. 2010
A. praetervisum	URM 84223	Brazil	KT006605	-	Gomes-Silva et al. 2010
A. rude	CANB 643174	Australia	KU315197	KU315197	Costa-Rezende et al. 2016
A. rugosum	GACP14062120	Thailand	MK077648	-	This study
A. rugosum	GACP16072714	Laos	MK077647	-	This study
A. rugosum	GACP16072707	Laos	MK077646	-	This study
A. rugosum	GACP 14081118	Hainan, China	MK077644	-	This study
A. rugosum	GACP14081522	Hainan, China	MK077645	-	This study

Table 1 Continued.

Species	Voucher/ Strain	Origin	ITS	LSU	References
A. rugosum	Dai 10746	China	KU219981	-	Song et al. 2016
A. schomburgkii	URM 84228	Brazil	KT006608	-	Gomes-Silva et al. 2010
A. schomburgkii	URM 84254	Brazil	KT006611	-	Gomes-Silva et al. 2010
A. sessile	URM83905	Brazil	JX982570	-	Gomes-Silva et al. 2010
A. sprucei	FLOR 52184	Brazil	KU315201	-	Costa-Rezende et al. 2016
A. sprucei	FLOR 52191	Brazil	KU315200	KU315216	Costa-Rezende et al. 2016
A. subsessile	URM 83239	Brazil	JX310860	JX310860	Gomes-Silva et al. 2010
A. subsessile	URM 83905	Brazil	JX982570	-	Gomes-Silva et al. 2010
A. yunnanense	Cui 7974	Yunnan, China	KJ531653	KU220013	Li & Yuan 2015
A. yunnanense	Dai 13021	Yunnan, China	KJ531654	-	Li & Yuan 2015
Tomophagus colossus (Fr.) Murrill	TC02	China	KJ143923	-	Zhou et al. 2015

Results and Discussion

Phylogeny

The tree topologies obtained from ML and BY were identical. Therefore, only the ML tree is shown in Fig. 1. Two major clades; South American and South East Asian, were identified in *Amauroderma* (Fig. 1). *Amauroderma rugosum* sequences obtained from China (GACP14081118 and GACP14080952), Thailand (GACP1406212) and Laos (GACP16072714 and GACP14081522) clustered in a well-supported clade forming monophyletic group (BS=100%; BPP=1.0) (Fig. 1). Given the phylogenetic results obtained herein where our new collections are found in a clade with *A. rugosum*, we believe that it would taxonomically be more appropriate to establish them as new records of *A. rugosum*. Furthermore, the deep nodes are not supported well in the tree, but this does not affect the final conclusions of the study. However, to obtain a better view of evolution of the genus, a phylogeny with more genes, and in particular single-copy nuclear genes such as tef1 or rpb2 would be recommended.

Taxonomy

Amauroderma P. Karst., 1881, Rev. Mycol. (Toulouse) 3, p. 17.

= *Amauroderma* (Pat.) Torrend, Brotéria, sér. Bot. 18: 121 (1920)
= *Ganoderma* sect. *Amauroderma* Pat., Bull. Soc. mycol. Fr. 5(2, 3): 75 (1889)
= *Lazulinospora* Burds. & M.J. Larsen, Mycologia 66(1): 97 (1974)
= *Magoderna* Steyaert, Persoonia 7(1): 111 (1972)
= *Whitfordia* Murrill, Bull. Torrey bot. Club 35: 407 (1908)

Description (from Ryvarden 2004)

Basidiocarps annual or reviving for a second season, centrally-laterally stipitate, solitary or in small groups with several fused pilei, consistency coriaceous, corky to woody hard, seldom brittle.

Pileus round, reniform to fan-shaped, concave, umbilicate to strongly infundibuliform, upper surface in varying colours from white, ochraceous, brown to almost black, finely tomentose to glabrous, dull to glossy with a distinct cortex or cuticle, often concentrically zoned and radially wrinkled, stipe rather thin and long, finely tomentose to glabrous, pore surface whitish to ochraceous when fresh, darkens when dry to brownish colours, pores round to angular and entire, large to small, tubes seldom stratified, context white, ochraceous to dark brown, cystidia absent, hyphal system dimitic, generative hyphae with clamps hyaline and thin-walled, skeletal hyphae arboriform to more rarely unbranched, hyaline to brown, those being hyaline often dextrinoid or without reaction in Melzer´s reagent, basidia bladder-like with 4 large curved sterigmata, basidiospores hyaline to pale yellow, sub-globose to cylindrical, dextrinoid to non-dextrinoid, bitunicate with the inner wall finely asperulate or very rarely smooth.

Type species: *Amauroderma regulicol*or (Berk. ex Cooke) Murrill (= *Amauroderma schomburgkii*)

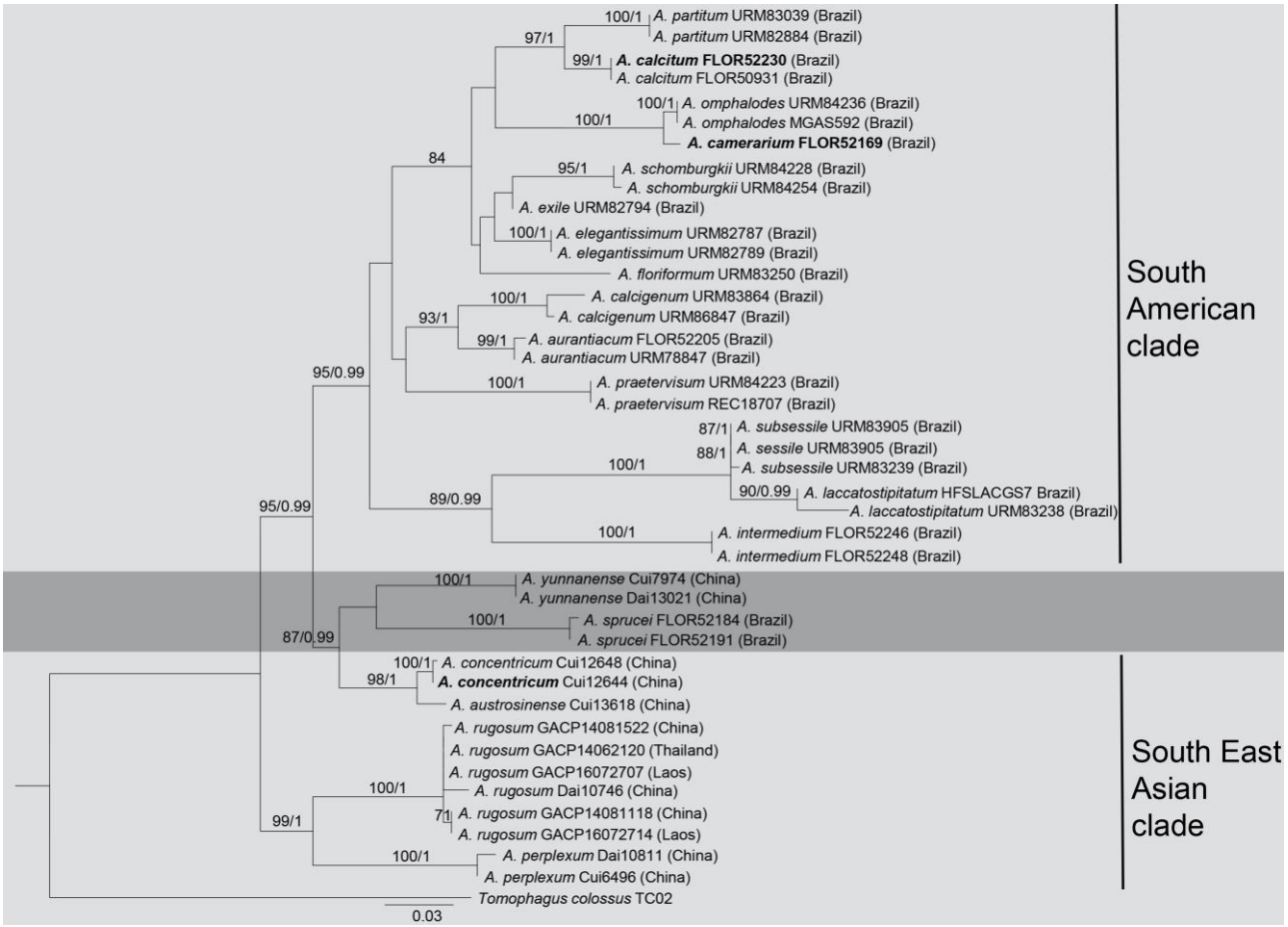

Fig. 1 – Phylogram generated from maximum likelihood analysis of ITS and LSU sequence data. Bootstrap support values for maximum likelihood, greater than 70% and Posterior Probabilities from Bayesian Inference ≥ 0.95 are given above branches. The tree is rooted with *Tomophagus colossus*. The strain numbers and the countries of origin are mentioned after the species. Type species are indicated in black bold.

Amauroderma preussii (Henn.) Steyaert, Persoonia 7(1): 107 (1972) Fig. 2

≡ *Ganoderma preussii* Henn. (1891)

(See Index Fungorum for other synonyms)

Facesoffungi number. FoF05185

Basidiome annual, corky, with distinctly contracted base at the center, becoming hard corky to woody hard when dry. *Pileus* single, 8–12 cm, up to 1 cm thick at the base, orbicular; upper

surface brown (6E4) to dark brown (6E5) alternating colour zones, near to margin, weakly laccate to non laccate, concentrically undulate, radially rugose; margin grey (6F1), wavy, inflexed; lower surface usually brown (6D7). *Hymenophore* up to 10 mm long, indistinctly stratose; pores initially brownish orange (5C4), bruising brown (6E8), pores circular or isodiametric, 2–4 per mm. Context 1 cm thick, triplex, not completely homogeneous in color; lower layer whitish yellow (4A2) to yellow (4A3), corky; middle layer whitish yellow (4A2) to yellow (4A3); fibrous/pithy, composed of coarse loose fibrils; upper layer yellowish brown (6B3), woody. *Basidiospores* (n = 25) (8.5)9.0–*9.9*–10.8(12.1) × (6.7)7.6–*8.5*–9.5(10.5) µm (Q_m = 1.1, Q = 0.9–1.4, with myxosporium). (n = 25) (7.5)7.9–*8.6*–9.4(10.1) × (4.9)6.2–*7.3*–8.3(9.9) µm (Q_m = 1.1, Q = 0.8–1.6, without myxosporium), subglobose, bitunicate, with a dark brown (6D8), eusporium bearing echinulae, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, pale yellow (4A3), streaks near the cutis, a closely-packed palisade, whitish yellow (4A2), clavate terminal elements. *Context* trimitic; generative hyphae (n = 30) (0.3–*0.9*–1.6) µm in width, colorless, thin-walled; skeletal hyphae (n = 30) (1.9–*2.4*–3.1) µm in width, thick walled, sometimes branched brown (6E4) to greyish brown (6E3); binding hyphae (n = 30) (0.4–*1.4*–2.3) µm in width, branched, with clamp-connections, brown (6E4) to greyish brown (6E3), frequently branched at apex, intertwined with the skeletal hyphae (Fig. 2).

Habitat – Rotten conifer wood, on the soil near humus rich soil with over heavily rotted litter on the ground, growing up from soil.

Specimens examined – Laos, Xiengkhouang Province, Phoukoud District, Yai village, evergreen forest, 19°58′N-103° 00′E, elev. 1120 m, collection date 27 July 2016, collector P. Phengsintham (GACP16072703, GACP16072833). China, Hainan Province, Jiangfengling Mountain, Coniferous rainforest, 18°44′N-108°51′E, elev. 550 m, collection date 21 October 2015, collector X.L Wu (GACP WXL15100201).

Notes – *Amauroderma preussii* was introduced as *Ganoderma pressuii* by Hennings (1891) from Cameroon. Steyaert (1972) transferred this species to *Amauroderma* (as *A. pressuii*). This species is characterized by sub-circular to circular, concentrically undulate pileus with darker concentric shades and spherical basidiospores (Steyaert 1972). Macroscopically, *A. preussii* is very difficult to distinguish from *A. oblongisporum* and the crust of the pileus of this species is thicker than in *A. preussii* and it is smoother in dry condition (Ryvarden & Johansen 1980). Furthermore, the pileus of this species is similar to *A. wuzshishanense*, however the latter has deep funnel-shaped pileus and larger spores (Wu & Dai 2005). This species has been recorded previously from African countries as a wood decaying species (Steyaert 1972, Ryvarden & Johansen 1980). It was recorded from various parts of China as a wood inhabiting polypore species (Bi et al. 1993, Wu & Dai 2005, Dai et al. 2011). Our collections from Laos agree well with description provided by the Ryvarden & Johansen (1980), Bi et al. (1993), Wu & Dai (2005) even though we were unable to obtain the DNA. Furthermore, there is not any sequence data available for *A. preussii* in GenBank

Amauroderma rugosum (Blume & T. Nees) Torrend Brotéria, sér. bot. 18: 127 (1920) Fig. 3

≡ *Polyporus rugosus* Blume & T. Nees 1826

= *Amauroderma amoiense* J.D. Zhao & L.W. Hsu, Acta Mycol. Sinica 2: 164. (1983)

= *Amauroderma wuzhishanense* J.D. Zhao & X.Q. Zhang, Acta Mycol. Sinica 6: 208. (1987)

(See Index Fungorum for other synonyms)

Facesoffungi number. FoF05186

Sanctioning author:

Fr.

Basidiome annual, stipitate, weakly laccate, corky. *Pileus* 2.5–3.6 × 2.0–2.4 cm, up to 0.5 cm thick at the base, subreniform, mesopodal; upper surface brownish orange (6C4) to brown (6E8), radially rugose, concentrically sulcate with irregularly ruptured crust, wrinkled towards the edge;

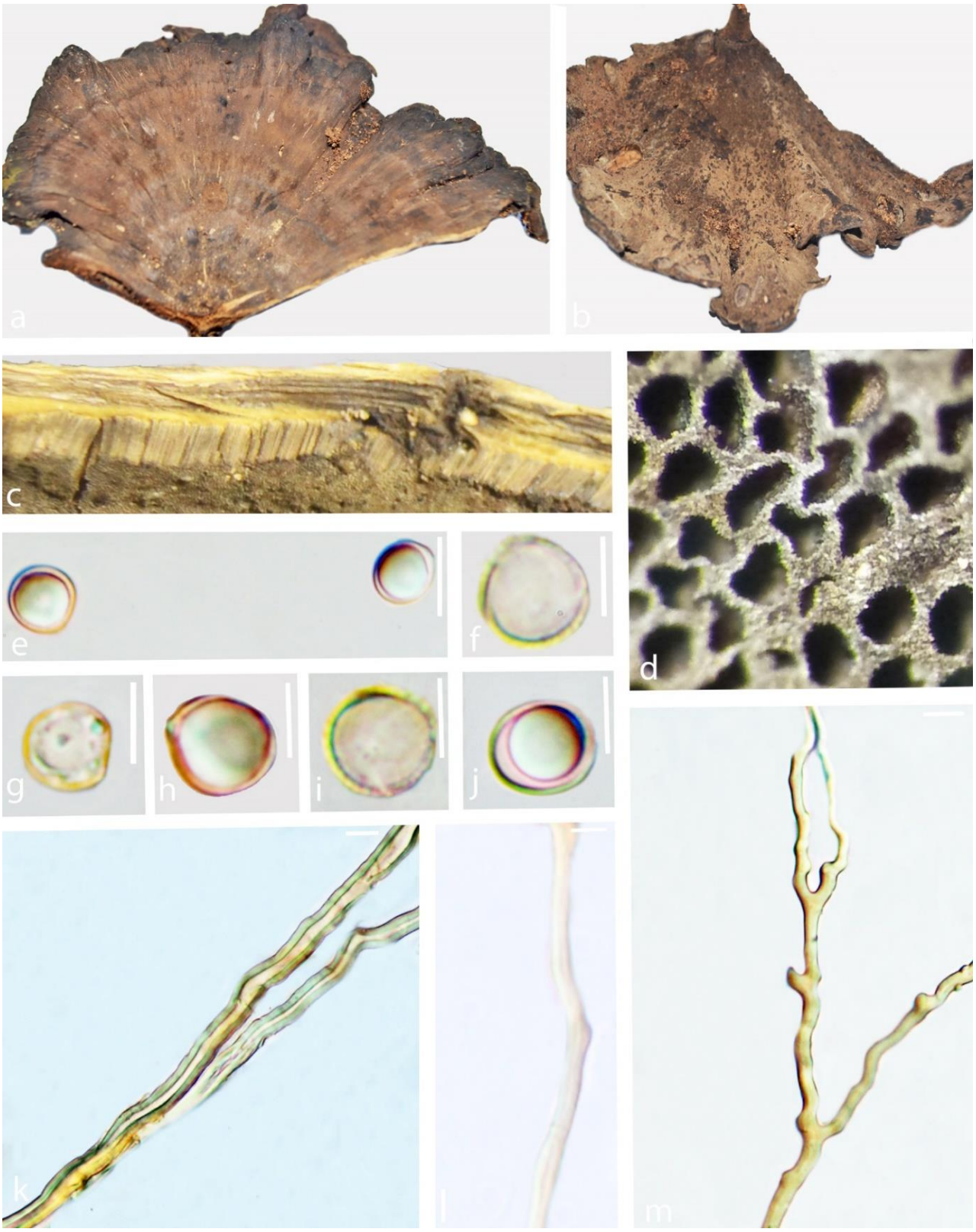

Fig. 2 – *Amauroderma preussi* (GACP16072703). a Upper surface. b Lower surface. c Pores in the lower surface (5×). d Cut surface. e–j Spores (100×). k Skeletal hyphae (100×). l Generative hyphae (100×). m Binding hyphae (100×). Scale bars: e–j = 10 μm, k–m = 5 μm.

margin blunt or wavy, concolorous with the pileus; lower surface greyish orange (5B5). *Hymenophore* up to 12 mm long, indistinctly stratose; pores initially brownish orange (5C4), bruising brown (6E8), pores circular, 3–5 per mm. Context up to 8 mm thick, duplex, dry; upper layer light brown (5D6), fibrous, composed of coarse loose fibrils; lower layer brown (6E8), corky.

Stipe eccentric, sub cylindrical, concolorous with the pileus, 5 × 7 cm. *Basidiospores* (n = 20) (9.5–)10.1–*10.7*–11.6(11.7–) × (–7.7)7.9–*8.5*–9.4(–9.6) µm (Q_m = 1.2, *Q* = 1.1–1.4, with myxosporium). (7.5–)8.3–*9.3*–10.4(–10.6) × (5.6–)5.8–*6.9*–8.0(–8.4) µm (Q_m = 1.2, *Q* = 0.9–1.3, without myxosporium), subglobose, brownish yellow (5B3), with a brown eusporium, overlaid by a hyaline myxosporium. *Pileipellis* a hymeniderm, brownish orange (5C4), clavate like cells, dextrinoid. Context trimitic; generative hyphae (n = 25) (0.5–*1.1*–2.2) µm, hyaline, thin–walled with clamp connections, rarely seen; skeletal hyphae (n = 25) (1.2–*2.5*–3.5) µm, thick–walled, nearly solid, sometimes branched, ochre orange white (5A2); binding hyphae (n = 20) (0.6-*1.7*-2.6) µm, thick-walled, branched, nearly solid, orange white (5A2) (Fig. 3).

Habitat – Rotten conifer wood, in dry dipterocarp forest and in upper mixed deciduous forest, growing up from soil.

Specimens examined – Laos, Xiengkhouang Province, Phoukoud District, Yai village, evergreen forest, 19°58′N-103°00′E, elev. 1120 m, collection date 27 July 2016, collector P. Phengsintham (GACP16072707, GACP16072714). Savvanakhet province, Phin district Phouxang Hae Protected Area, mixed deciduous forest, $16^0$58′N -$105^0$89′E, elev. 173 m, collection date 23 June 2017, collector P. Phengsintham (GACP17062326, GACP17062328), China, Hainan Province, Jiangfengling Mountain, Coniferous rainforest, 18°44′N-108°51′E, elev. 550 m, 19°12′N 109°42′"E, collection date 9 August 2014, collector T.C Wen (GACP14080910, GACP14080952, GACP14080929, GACP14080956, GACP 14081118, GACP14081522). Thailand, Chiang Mai Province, Mushroom Research Center, Coniferous rainforest, 19°20′N-98°44′E, elev. 770 m, collection date 2014/06/21, collector LS Zha (GACP14062120, GACP14062122, GACP14062124).

Notes – Blume & T. Nees described this species as a *Polyporus rugosus* on the basis of specimens from Java in 1826. Berkeley (1856) introduced a new species *Porothelium rugosum* from Brazil and Steyaert (1972) suggested *Polyporus rugosus* and *Porothelium rugosum* were similar species based on their morphological descriptions. *Polyporus rugosus* was transferred to *Ganoderma* (as *G. rugosum*) by Patouillard (1889). Patouillard (1894) took up the name *Porothelium rugosum* Berk. and changed it as *Ganoderma sprucei* Pat., since he considered that there were already an epithet called "rugosum" in *Ganoderma*. Torrend (1920) transferred *G. sprucei* Pat. to *Amauroderma* (*A. rugosum*). *Porothelium rugosum* was synonymized Berk. As *Amauroderma sprucei* (Pat.) Torrend (Furtado 1968). Thus, it was a problem whether *P. rugosum* Berk. (= *G. sprucei* Pat.) and *G. rugosum* (BI. & Nees) Pat. were similar species. Torrend therefore continued to use 'sprucei', the earliest epithet available in *Amauroderma*. Considering the combination of *Porothelium rugosum* in *Foraminispora* the epithet is available. However, Costa-Rezende et al. (2017) proposed *Foraminispora*, a new genus to accommodate *Porothelium rugosum* (= *Amauroderma sprucei*) based on strong morphological and molecular data. According to Index Fungorum and MycoBank, *A. rugosum* (Blume & T. Nees) Torrend is now a legitimized species characterized by mesopodal and often excentric, or often pleuropodal pileus with subglobose basidiospores. *Amauroderma rude* (Berk.) Torrend also has a light context and black pileus, but the pores are larger (2–3 per mm), and it is mainly distributed in Australia (Cunningham 1965). Ryvarden & Johansen (1980) provided detailed description of this species and our collections agree well with that description and moreover, the descriptions provided by Chinese authors (Teng (1963, Tai 1979, Zhao et al. 1981, Zhao 1989, Bi et al. 1993, Teng 1996, Zhao & Zhang, 2000). This species is a soil-inhabiting saprobe and usually found on the ground or attached to buried roots in hardwood forests (Baran De 1991) and widely distributed in the tropics, especially in South East Asia. In East Asia it is known from subtropical China, Japan, Taiwan, Northern Thailand, and Vietnam (Núñez & Ryvarden 2000, Hapuarachchi et al. 2018b).

Beneficial medicinal properties of *Amauroderma*

Previously described members of Ganodermataceae have a long history of use to promote health and longevity in Asia (Hapuarachchi et al. 2017). Its species are widely researched,

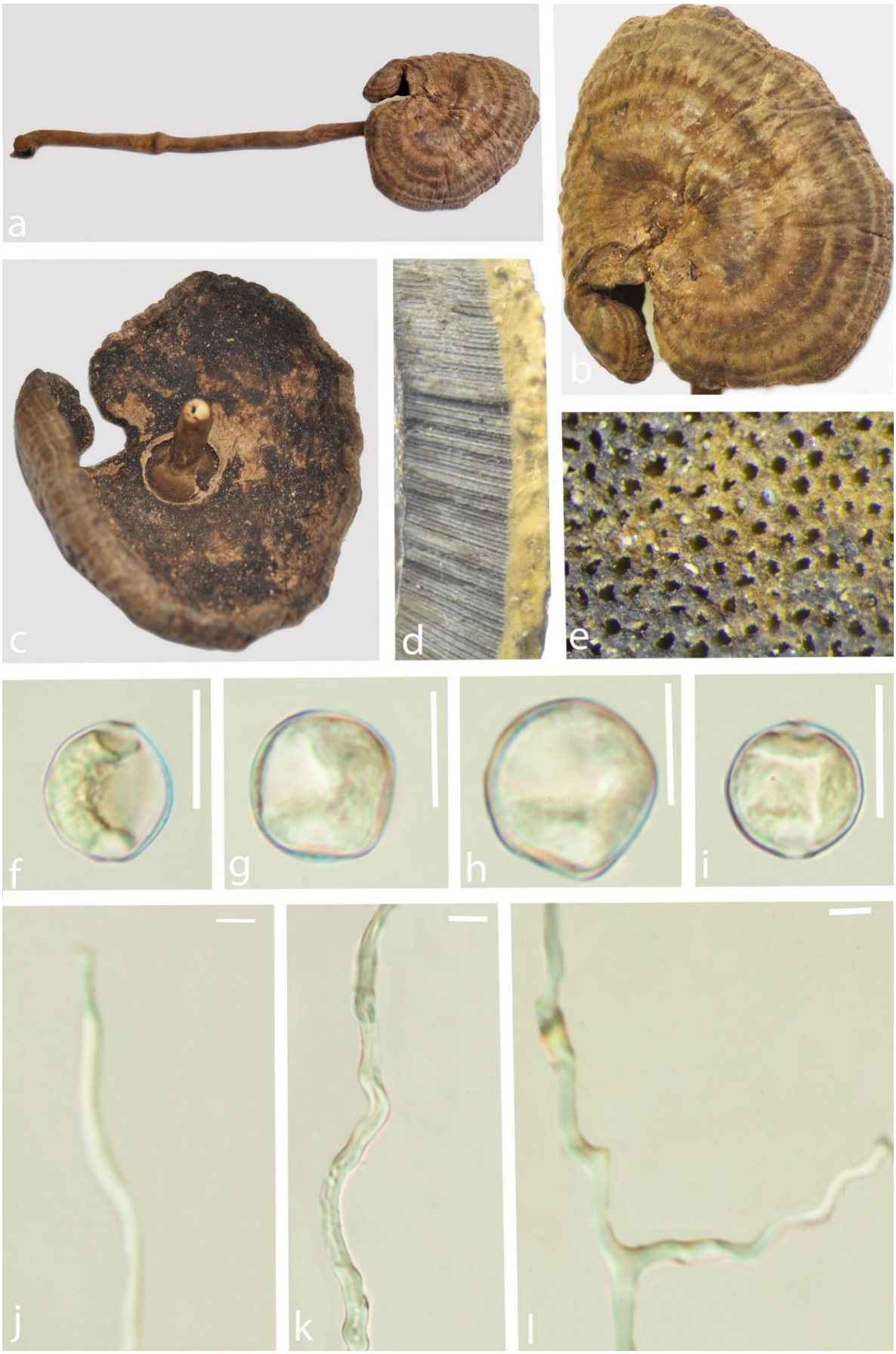

Fig. 3 – *Amauroderma rugosum* (GACP16072714). a, b Upper surface. c Lower surface. d Cut surface. e Pores in the lower surface (5×). f–i Spores (100×). j Generative hyphae (100×). k Skeletal hyphae (100×). l Binding hyphae (100×). Scale bars: f–i = 10 μm, j–l = 5 μm.

because of their highly prized medicinal value with many chemical constituents with potential nutritional and therapeutic values (Hapuarachchi et al. 2016a, 2016b, Hapuarachchi et al. 2018a). Species in *Amauroderma* have been newly recognized as medicinal fungi (Chan et al. 2013, Jiao et al. 2013, Zhang et al. 2013). *Amauroderma* species are commonly known as the "epileptic child mushroom" or "Jia zhi" in China. It is traditionally used by the Chinese to reduce inflammation, to treat diuretic and indigestion, and to prevent cancer (Dai & Yang 2008). The indigenous Temuan people in Malaysia believe this fungus has a power to heal epilepsy (Chang & Lee 2004, Azliza et al. 2012). It was suggested that volatile components which may be present in the mushroom, may have contributed to the beneficial effects of this mushroom (Chan et al. 2015). In the following part of this paper, we discuss various bioactive compounds produced by *Amauroderma* species and its beneficial medicinal properties.

A new triterpenoid compound named amauroamoienin, together with 13 known compounds from ethyl acetate extracts of *Amauroderma amoiensis* (= *A. rugosum*) were discovered by Zhang et al. (2013). Amauroamoienin, (l7R)-17-methylincisterol and jacareubin compounds exhibited acetyl cholinesterase inhibitory activities (Zhang et al. 2013). Purified Amaurocine, a purified novel protein from *Amauroderma camerarium* fermentations showed Amaurocine's activity against *Trichomonas vaginalis* isolates. It is the causative agent of Trichomoniasis, the most common nonviral sexual transfer disease worldwide. Furthermore, this protein demonstrated low toxicity towards human neutrophils and a pro-inflammatory character and Amaurocine may produce a synergic action being directly cytotoxic against the parasites and indirectly enhancing the host immune response, improving the protection from this mucosal pathogen (Duarte et al. 2016).

Twelve new compounds were isolated from the fruiting bodies of *Amauroderma rude* (diptoindonesin D, 6-deoxyjacareubin, jacareubin, 1H-indole-3- carboxylic acid, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenylethanol, 3β-hydroxy-7,22E-dien-ergosta, 3β,7α-dihydroxy-8,22E-5α,6α-epoxyergosta, 3β-hydroxy-7α-methoxy-8(14),22E-dien-5α,6α-epoxyergosta, ergosterol 5α,8α- peroxide, 3β-5β-8β-trihydroxy-6,22E-ergosta, and 3β,5α-6β-trihydroxy-7,22E-dien-ergosta). Among them 6-deoxyjacareubin and jacareubin exhibited the cytotoxic activities against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines and 3β-hydroxy-7α-methoxy-8(14), 22E-dien-5α, 6α-epoxyergosta showed the cytotoxic activities against HL-60, MCF-7, and SW-480 cell lines (Chen et al. 2016). Low concentrations of water extracts of *A. rude* could inhibit breast cancer cell survival and induce apoptosis Jiao et al. (2013). Pan et al. 2017) revealed that oral administration of *A. rude* extract daily for 90 days does not cause any subchronic toxicity of mice. Li et al. (2015) found, ergosterol purified from *A. rude* induced cancer cell death *in vivo*. Furthermore ergosterol-mediated suppression of breast cancer cell viability occurred through apoptosis and that ergosterol up-regulated expression of the tumor suppressor Foxo3. They suggested ergosterol is the main anti-cancer ingredient in *A. rude*, which activated the apoptotic signal pathway. Pan et al. (2015) showed crude *A. rude* extract under *in vitro* experiments showed the capacities of spleen lymphocytes, macrophages, and natural killer cells were increased in tumor growth and *in vivo* experiments showed the extract increased macrophage metabolism, lymphocyte proliferation, and antibody production. Furthermore, the partially purified product stimulated the secretion of cytokines *in vitro*, and *in vivo* and in turn decreased tumor growth rates. The active compound was purified and identified as polysaccharide F212 and it had the highest activity in increasing lymphocyte proliferation. Wang & Qi (2016) concluded that the extract of *A. rude* - roots of *Lentinus* solid fermentation compounds possessed antioxidant activity. Ten chemical compounds were isolated from the fruiting bodies of *A. subresinosum* including 2 new ones named amaurosubresin and erythro (23, 24)-5α, 6α, epoxyergosta-8-ene-7-one-3β, 23-diol. The bioassay of inhibitory activity against acetyl cholinesterase (AChE) of two new isolates exhibited definite inhibitory activity (Wang et al. 2016).

The presence of carbohydrates, proteins, dietary fibre, phosphorus, potassium, and sodium in mycelia of *Amauroderma rugosum* has been confirmed by Chan et al. (2013). Furthermore, *A. rugosum* Ethyl acetate extract exhibited the highest total phenolic content and the strongest antioxidant activity based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. Hexane extracts showed dose-dependent inhibition of NO production in LPS-stimulated RAW264.7 cells and NO radical scavenging activity. In addition Gas chromatographic analysis of Hexane extracts showed the presence of ethyl linoleate and ergosterol, compounds with known anti-inflammatory properties. Hence, mycelia extracts of *A. rugosum* have the potential to serve as a therapeutic agent or adjuvant in the management of inflammatory disorders Chan et al. 2013). Wild and domesticated basidiocarps of *A. rugosum* possessed anti-oxidant activity and *in vitro* anti-inflammatory properties. Ethanolic extractions of wild and domesticated basidiocarps inhibited downstream inflammatory mediators (TNF-α and NO) and induced anti-inflammatory cytokine IL-10 production. No inhibitory effects shown on upstream nuclear translocation of NF-κB p65. Furthermore, both wild and domesticated ethanolic extractions exhibited antioxidant activity and attenuation of proinflammatory mediators (Chan et al. 2015).

Amauroderma rugosum showed antimicrobial activity against *Staphylococcus aureus, S. pyogenes, Pseudomonas aeruginos, Escherichia coli* and *Clostridium difficile* (Liew et al. 2015). Methanol and cold and hot water extracts of the freeze-dried mycelial culture of *A. rugosum* exhibited no or little cytotoxic effect against the MCF-7 and A-549 cell lines. Furthermore, oral administration of a single dose of mycelial powder (2000 mg/kg) to Sprague-Dawley rats had no adverse effect on the growth rate or hematological and clinical biochemical parameters and not induce any pathological changes in the organs of the tested animals (Fung et al. 2017). Oleate-induced HepG2 cells treated with *A. rugosum* ethyl acetate (EA) extract greatly decreased intracellular and secreted total triglyceride (TG) and total cholesterol (TC) compared with other extracts. Hence, the *A. rugosum* EA extract is a good source of lipid-ameliorating agents in the management of dyslipidemia (Seng et al. 2017a). The semipolar ethyl acetate (EA) fraction of *A. rugosum* demonstrated good antioxidant capacity based on total phenolic content, 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging, ferrous ion-chelating ability, cupric ion-reducing antioxidant capacity, and lipid peroxidation assays. The EA fraction also showed the strongest inhibitory effect on Cu2+-induced LDL oxidation via thiobarbituric acid reactive substances formation and HMG-CoA reductase activity. Moreover, the phenolic compounds (4 benzoic acid derivatives, 3 flavonoids, 1 cinnamic acid, 1 hexahydroxydiphenic acid dilactone, and 1 xanthone derivative), play pivotal roles in arresting the physiopathogenesis of atherosclerosis and attenuating the risk of cardiovascular events occurring (Seng et al. 2017b).

Research on various metabolic activities of *Amauroderma* have been performed *in vitro* studies. However, there has been no report of *in vivo* studies and human trials using *Amauroderma* as a direct control agent diseases. Hence, *Amauroderma* and related products can be used as a therapeutic drug if more direct scientific evidence are available in the future.

Conclusion

Macroscopic, microscopic, and molecular data all confirm that the *Amauroderma* collections from Laos belong to *Amauroderma pressuii* and *A. rugosum*. This is the first discovery of these two species in Laos. The studies of more collections of these species are needed to better estimate the variability of these taxa. Some *in vitro* studies of medicinal properties of *Amauroderma* appear to be promising, but careful investigation and accurate scientific evidences needed for establishing the safe and efficient use of *Amauroderma.* Experimental, epidemiological, and clinical studies should be carried out on identification of the molecular targets and investigate the association between *Amauroderma* intake and disease risk.

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