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The genus *Lentinus* in Thailand: taxonomy, cultivation tests, nutritional analysis and screening for the biological activity of wild strains

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Abstract

This is a case study of *Lentinus* in northern Thailand, including taxonomy, fruiting tests, nutritional analysis and screening for the biological activity of wild *Lentinus* strains. Five species (*L. arcularius, L. badius, L. polychrous, L. sajor–caju* and *L. squarrosulus*) were investigated based on morphology and a comprehensive study detailing habitat, distribution; and further including phylogenetic analyses based on ITS and LSU; and finally, each species was verified. Three active wild strains (*L. arcularius, L. sajor–caju,* and *L. squarrosulus*) were selected to observe the optimal media, temperature, and pH for mycelial growth. In addition, optimum cereal media for spawn production was studied. Four wild strains of *L. squarrosulus*, one strain of *L. sajor–caju,* and two strains of *L. arcularius,* were cultivated on sawdust substrate, and they were observed for fruiting body production, fresh weight, and biological efficiency. Extracts from *L. sajor–caju* and *L. squarrosulus* have been made to measure nutritional values, which comprise protein, fiber, carbohydrates and fat. Furthermore, *L. sajor–caju* and *L. squarrosulus*, were screened for biological activity for inhibition of alpha–glucosidase enzyme activity. These results on wild strains of *Lentinus* will be useful for further studies on the applications of *Lentinus*.

Keywords – biological activity – cultivation – diversity – enzymes – *Lentinus* – nutritional values – screening

Introduction

Lentinus Fr. is a lamellate and poroid hymenophore genus of Polyporaceae Fr. ex Corda, consisting of 55 species worldwide, which are well known as wood rot and white rot to decay hardwood (He et al. 2019). *Lentinus* is characterised as having lentinoid or polyporoid basidiomata with white to brown fibrillose, sarcomonomitic or sarcodimitic hyphal system, clamp–connections in generative hyphae, dendroid skeletal hyphae (skeletal–ligative hyphae) and hyaline to rusty–brown pigments, presence of peudocystidia in subgenus *Lentinus*, and clavate basidia with 4–spores (Zmitrovich 2018). They are distributed in both tropical and temperate regions, but most species are in tropical habitats (Seelan et al. 2015). *Lentinus* was placed in Tricholomataceae R. Heim ex Pouzar by considering their pileus, lamellae and white spores (Miller 1973). Later, the genus was considered to have a closer relationship to Polyporaceae because of their complicated characters in

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the hymenium layer observed as being similar to Polypolaceae (Corner 1981, Pegler 1983). Moreover, molecular evidence and morphology of *L. tigrinus* (Bull.) Fr. pointed to a greater similarity to Polyporaceae than Tricholomataceae (Hibbett & Vilgalys 1991). In addition, the analysis of nuclear–encoded ribosomal RNA genes (rDNA) strongly suggested that all *Lentinus* species are more closely related to Polypore than Agarics (Hibbett & Vilgalys 1991). Furthermore, three poroid hymenophore species, *L. arcularius* (Batsch) Zmitr., *L. brumalis* (Pers.) Zmitr. and *L. tricholoma* (Mont.) Zmitr. were placed in *Lentinus*; and *Lentinus* subgen. *Lentinus* has been devided into 5 sections, which are sect. *Dicholamellatae* Pegler, sect. *Lentinus* sensu Pegler, sect. *Lentodiellum* (Murr.) Pegler, sect. *Rigidi* Pegler, sect. *Tigrini* Pegler; and there are two clades of *Poreporelles* (angler and circular) (Seelan et al. 2015, Zmitrovich & Kovalenko 2016). Later, Zmitrovich & Kovalenko (2016) and Zmitrovich (2018) divided *Lentinus* into two subgenera; which are *Lentinus* subgen. *Lentinus* Fr. with two sections (Sect. *Lentinus*, sect. *Tigrini*), and subgenus *Polyporellus* (P. Karst.) Zmitr with seven species; which are *L. arcularius*, *L. brumalis*, *L. ferruginipes* (Corner) Zmitr, *L. flexipes* (Fr.) Zmitr. et Kovalenko, *L. longiporus* (Audet, Boulet et Sirard) Zmitr. et Kovalenko, *L. substrictus* (Bolton) Zmitr. et Kovalenko, *L. tricholoma* and *L. vossii* (Kalchbr.) Zmitr. et Kovalenko. However, *Lentinus* is not a monophyletic genus.

In Thailand, *Lentinus* species fruit well in the early rainy season when the weather is hot and humid while some species can be found year–round regardless of season (Sysouphanthong et al. 2010). Some species found in Thailand include *L. arcularius* (Batsch) Zmitr. (= *Polyporus arcularius* (Batsch) Fr.), *L. concinnus* Pat., *L. connatus* Berk., *L. badius* (Berk.) Berk., *L. fasciatus* Berk., *L. squarrosulus* Mont., *L. polychrous* Lev., *L. cladopus* Lev., *L. retinervis* Pegler, *L. sajor–caju* (Fr.) Fr., *L. strigosus* (Schwein.) Fr., *L. stuppeus* Klotzsch, *L. swartzii* Berk., *L. tigrinus* (Bull.) Fr., *L. zeyheri* Berk (Chandrasrikul et al. 2011). However, these studies were only based on morphology. In the latest study, Karunarathna et al. (2011) described three new species of *Lentinus* from Thailand based on molecular evidence and morphology, which are *L. roseus* Karun., K.D. Hyde & Zhu L. Yang, *L. concentricus* Karun., K.D. Hyde & Zhu L. Yang and *L. megacystidiatus* Karun., K.D. Hyde & Zhu L. Yang. Unfortunately, we did not find these examples in this study, and those species seem to be members of *Panus* because of their presence of skeletal hyphae.

Most *Lentinus* are edible and have been described to have medicinal properties, while some species have been cultivated for consumption and medicinal uses worldwide (Adesina et al. 2011, Fasidi & Kadiri 1993, Okhuoya et al. (2005). However, only *L. squarrosulus* and *L. polychrous* are currently cultivated in Thailand (Thawthong et al. 2014). Klomklung et al. (2014) studied the growth of mycelium of Thai strains of *L. conatus* and *L. roseus* in different media, and it was found that both strains grew well in black bean agar and red bean agar, at 30 °C, with a pH of 5.0–7.0. Afiukwa et al. (2015) found that *L. conatus* has a higher protein and amino acid contents than *Auricularia auricula–judae* (Bull.) Quél. Some studies have reported bioactive compounds found in *Lentinus*, and it was found that *L. sajor–caju* and *L. tigrinus* have antioxidant activities (Dulay et al. 2015), and the ethanolic component of *L. polychrous* also has anti–inflammatory effects (Fangkrathok et al. 2013).

Lentinus squarrosulus is an important species and is cultivated in tropical countries (Lau & Abdullah 2016). In Thailand, it is a natural food source from forests in the wet season and is grown for consumption throughout the year (Thawthong et al. 2014, Pukahuta et al. 2008, Sakaew et al 2013). For more details of species, a revision of utilisation, nutritional attributes, bioactive compounds, biological activities and antioxidant capacity are given by Lau & Abdullah (2016). We observed that *Lentinus polychrous* and *L. sajor–caju* are sold in markets in Thailand, but we did not find evidence of cultivation. These two species are probably useful for cultivation and consumption.

Previous studies of *Lentinus* in Thailand were ambiguous in terms of taxonomy and species names. In this study; we aim to identify *Lentinus* species in Thailand based on both morphology and molecular data; find out the suitable medium, temperature, and pH for mycelial growth, spawn production and fruiting body production of wild *Lentinus* strains; and analyze the nutritional content and screen the biological activity of selected *Lentinus* species.

Materials & Methods

Taxonomy

Mushroom collection and examination

Samples were collected during the wet season (June to October) of 2018–2019 in northern Thailand. General information included collecting date, and recording GPS of locality, forest type, soil type, and habitat. Morphological characteristic notes were taken as previously described by Vellinga (1988). Colours of mushrooms were defined based on the colour book of Kornerup & Wanscher (1978). The samples were dried in a food dryer (50 °C) for 12–24 hours, preserved in plastic bags, and deposited to the herbarium at Mae Fah Luang University (MFLU).

Microcharacteristic study was performed as described in Karunarathna et al. (2011). Line drawings of microscopic characters were made using a drawing tube attached to an Olypus CX–41 research compound microscope. A full description of each species was compared with available literature on *Lentinus* in tropical countries. Naming of fungi and nomenclature were performed following the Index Fungorum (2023). All records were added to the GMS database (Chaiwan et al. 2021).

Phylogenetic study

DNA was extracted from dried herbarium collections, according to the instructions of the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China). Primers ITS1 and ITS4 were used for the nrITS1, 5.8S and nrITS2 regions, primers LR0R and LR5 for the large subunit region; and PCR conditions were followed as previously described (Gardes & Bruns 1993, White et al. 1990). The PCR amplified products were cleaned and sequenced by Shanghai Majorbio Bio–Pharm Technology Co., Ltd. Sequences were edited and contigs were assembled using the SeqMan program (DNAStar, Madison, WI, USA), and then all new sequences were deposited in the GenBank. The sequences of new taxa were blasted using the website of The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), and some related sequences of each species were obtained and combined with new sequences in this study. The datasets were aligned using MAFFT version 7.130–win32 (Katoh et al. 2002, Katoh & Toh 2008). A Maximum likelihood (ML) analysis was performed in RAxML 7.2.6 (Stamatakis 2006), with GTRGAMMAI as the model of evolution, and branch support was estimated for over 1,000 bootstrap partitions (BP) with the rapid bootstrap option. Maximum parsimony (MP) analysis was performed using the program PAUP* 4.0 b10 (Swofford 2002); and the settings were 1,000 heuristic searches, employing TBR branch swapping and random sequence addition; gaps were treated as missing; all characters are of type unordered and all equally weighted; multistate taxa are interpreted as uncertainty; starting trees were obtained via stepwise addition; one tree was held at each step during stepwise addition; the steepest descent option was not in effect; branches were collapsed (creating polytomies) if minimum branch length was zero and MulTrees option was in effect. Bootstrap support BS values were calculated using 1,000 BS replicates, with 10 heuristic searches per replicate, random sequence addition, and TBR branch swapping. Branch support was estimated over 1000 bootstrap partitions (BP) with the rapid bootstrap option. The Bayesian inferences (BI) analysis was conducted using MrBayes on XSEDE 3.2.7a (CIPRES) and run one million generations for the dataset. The best substitution model was defined in MrModelTest v.2.3, and the best–selected model was HKY + I + G for ITS and GTR + I + G for LSU (Nylander 2004).

Table 1 List of specimens used in the phylogenetic analyses.

Species	Country	Specimen voucher no.	GenBank number	
			ITS	LSU
Ganoderma boninense	Japan	WD 2028	KJ143905	KU220015
Lentinus arcularius	Unknown	BAFC4535	KY706052	

Table 1 Continued.

Species	Country	Specimen voucher no.	GenBank number	
			ITS	LSU
L. arcularius	Argentina	CBS 325.49	MH856541	MH868066
L. arcularius	China	Cui10998	KX548973	KX548995
L. arcularius	China	Cui11398	KU189766	KU189797
L. arcularius	China	Cui12256	KX899966	KX900097
L. arcularius	China	Cui6450	KX899967	KX900098
L. arcularius	China	Dai 6756	KC572004	KC572043
L. arcularius	China	Dai 8159	KC572005	KC572044
L. arcularius	China	Li 1966	KX899970	KX900100
L. arcularius	Malaysia	DSH92132	KP283489	KP283522
L. arcularius	**Thailand**	**MFLU22–0020**	**OM780263**	**OM802485**
L. arcularius	**Thailand**	**MFLU22–0027**	**OM780264**	**OM802486**
L. arcularius	Japan	WD2138	AB478874	AB368081
L. arcularius	Japan	WD2359	AB478875	AB368082
L. badius	Thailand	DED07668	KP283480	KP283518
L. badius	Thailand	PU00436	KP283481	
L. badius	Malaysia	JSKT5858	KP283479	KP283513
L. badius	India	JZ4	MG273730	MG273730
L. badius	**Thailand**	**MFLU22–0029**	**OM780265**	
L. bertieri	Argentina	TFB10791	GU207305	
L. bertieri	Dominican Republic	TFB11723	GU207307	
L. brumalis	Belgium	CBS 370.34	MH855572	MH867078
L. brumalis	Belgium	CBS 368.34	MH855570	MH867076
L. brumalis	Belgium	CBS 369.34	MH855571	MH867077
L. brumalis	Belgium	CBS 371.34	MH855573	MH867079
L. brumalis	Russia	CBS 254.30	MH855135	MH866583
L. brumalis	China	Cui10750	KU189765	KU189796
L. brumalis	China	Cui2769	KX851595	KX851650
L. brumalis	China	Cui7188	KX851591	KX851646
L. brumalis	Japan	WD2372	AB478877	AB368084
L. ciliatus	Denmark	TENN57698	AB070882	AJ487943
L. ciliatus	China	Wei1582	KU189767	KU189798
L. crinitus	Puerto Rico	FPLM–PR2058	GU207295	KX065983
L. crinitus	Costa Rica	Tage_Roland_EF–229	GU207290	
L. crinitus	USA	TENN 59662	GU207289	
L. glabratus	Costa Rica	Tage_Roland_TR12	GU207250	
L. glabratus	Thailand	TP7	KF860882	
L. polychrous	Thailand	AH00024	KP283485	
L. polychrous	Thailand	BCC 29606	AB478882	LC052215
L. polychrous	Malaysia	JS0054	KP283486	
L. polychrous	Thailand	KM141387	KP283487	KP283514
L. polychrous	**Thailand**	**MFLU22–0030**	**OM780266**	**OM802487**
L. polychrous	**Thailand**	**MFLU22–0031**	**OM780267**	
L. sajor–caju	India	AO–DEBCR–4	KX342920	
L. sajor–caju	China	Dai 13712	KX900066	KX900181
L. sajor–caju	Tanzania	JMH36	KM267726	KM267733
L. sajor–caju	Malaysia	FRI62056	KP283492	KP283509
L. sajor–caju	Malaysia	JS0056	KP283494	KP283511
L. sajor–caju	Malaysia	SNP24989	KP283493	KP283510
L. sajor–caju	**Thailand**	**MFLU22–0032**	**OM780268**	**OM802488**
L. squarrosulus	India	AO–DEBCR–3	KT207470	KT459340
L. squarrosulus	Malaysia	FRIM4180	KP283483	KP283517
L. squarrosulus	Malaysia	BORH0009	KP283484	KP283515

Table 1 Continued.

Species	Country	Specimen voucher no.	GenBank number	
			ITS	LSU
L. squarrosulus	Japan	C500W	AB478883	LC052216
L. squarrosulus	China	CUI6513	KP283482	KP283516
L. squarrosulus	China	HFJAU0131	MN258658	
L. squarrosulus	Laos	HNL503448	OM802451	
L. squarrosulus	Laos	HNL503486	OM802452	OM802453
L. squarrosulus	**Thailand**	**MFLU22–0033**	**OM780269**	**OM802489**
L. squarrosulus	**Thailand**	**MFLU22–0034**	**OM780270**	**OM802490**
L. squarrosulus	**Thailand**	**MFLU22–0035**	**OM780271**	
L. squarrosulus	**Thailand**	**MFLU22–0036**	**OM780272**	**OM802491**
L. squarrosulus	**Thailand**	**MFLU22–0037**	**OM780273**	**OM802492**
L. striatulus	India	JSRK8	MK641478	
L. striatulus	India	NG9	KX580188	
L. striatulus	Costa Rica	Roland_MO135	GU207311	
L. tigrinus	Unknown	CBS 246.39	MH855998	MH867498
L. tigrinus	Unknown	CBS 248.39	MH856000	MH867500
L. tigrinus	Yugoslavia	CBS 249.39	MH856001	MH867501
L. tigrinus	Iran	IRAN279C	GU207255	AY615977
L. tigrinus	Belgium	MUCL22821	AB478881	AB368072
L. tricholoma	Unknown	BAFC4554	KY706061	KY706071
L. tricholoma	Unknown	CI53	KY706060	KY706070
L. tricholoma	USA	TENN 56503	AB478884	AB368100

Tests for cultivation, nutritional values, and screening of biological activities

Mushroom isolation

Fresh specimens from the collections were cleaned on the outer surface to avoid contamination from other microfungi. The pure tissue from the pileus or stipe was cut and transferred to PDA and then incubated at room temperature. After mycelium colonized well, it was subcultured in different media types, which were potato dextrose agar (PDA), potato sucrose agar (PSA), malt extract agar (MEA), oatmeal agar (OMA) and corn meal agar (CMA). The pure cultures were preserved in different media tubes and deposited in the MFLUCC herbarium. The FOF numbers for the taxa were obtained as instructed in Jayasiri et al. (2015).

Optimal media for mycelial growth

Three wild strains (*Lentinus arcularius, L. sajor–caju* and *L. squarrosulus*) were used for this experiment. Five types of media were used for the testing, namely potato dextrose agar (PDA), potato sucrose agar (PSA), malt extract agar (MEA), oat–meal agar (OMA) and corn meal agar (CMA). All media were autoclaved at 121 °C for 15 minutes and 20 ml of media were poured into each petri dish. The mycelial plug (0.5 cm diam.) of each mushroom strain was transferred to Petri dishes and incubated at 30 °C in the dark. Mycelial growth was evaluated by dry weight determination after 14 days. The media were melted and washed away with hot water, leaving the fungal mycelia. The growth of the mycelia was evaluated by the determination of dry weight. The experiment was carried out in triplicate.

Effect of temperature and pH

Potato sucrose agar (PSA) was used for *L. squarrosulus* (MFLU22–0033) and *L. sajor–caju* (MFLU22–0032) while oatmeal agar (OMA) was used for *L. arcularius* (MFLU22–0021). The agar media plates were inoculated by approximately 0.5 mm diameter of mycelium plug. The cultures of three strains of mushrooms were incubated at 20, 25, 30 and 40 °C for 12 days.

The optimal pH was evaluated in potato sucrose broth (PSB) for *L. squarrosulus* and *L. sajor–caju* while oatmeal broth (OMB) was used for *L. arcularius*. The media were adjusted to a pH of 2, 4, 6, 7, 8, and 10 with 1N HCl or 1N NaOH before autoclaving. The broth tube (50 ml) was then inoculated with three mycelial plugs approximately 0.5 mm in diameter. All cultures were incubated at 25 °C on a rotary shaker at 120 rpm in the dark. Mycelium growth was evaluated by determination of dry weight after 14 days. The experiment was conducted in triplicate.

Optimal grain/agricultural waste media for spawn production

Three wild strains were also tested for mycelial growth on four cereal media which include, sorghum (S), sorghum mixed with rice straw (SRS, 1:1 w/w), sorghum mixed with corn waste (SCW, 1:1 w/w), and sorghum mixed with rice husk (SRH, 1:1 w/w). Each strain was experimented with five replications. Each cereal medium was contained in glass tubes, and autoclaved at 121 °C for 15 minutes, three mycelial plugs of approximately 0.5 cm in diameter from the actively growing mycelia colony were transferred to each tube and incubated at 25 °C in a dark condition for 12 days.

Fruiting test

Pure cultures of *L. squarrosulus* (MFLU22–0033, MFLU22–0034, MFLU22–0035, MFLU22–0037 and NTF 228) *L. sajor–caju* (MFLU22–0032) and *L. arcularius* (MFLU22–0023 and MFLU22–0026) were used for this study. Rubber sawdust was used as the main substrate mixed (w/w) with 5% rice bran, 1% spent brewery grain, 1% of glutinous rice flour, 1% pumice sulfate, and 1% of calcium carbonate. All substrate supplements were manually mixed with 70% moisture and placed in polypropylene bags (800g). The bags were sterilized at 121 °C for 45 minutes.

After the temperature was reduced to room temperature, 50 g of the spawn of three strains of mushrooms were inoculated into sawdust bags under aseptic conditions. The bags were incubated at 25 ± 1 °C in the dark, for 60 days. For the fruiting phase, the same temperature and 75–85% humidity were used. Watering was carried out every morning and evening using a sprayer with tap water until the fruiting bodies had fully developed. Each strain was prepared with five replicates.

Nutritional analysis of cultivated Lentinus species

The nutritional analysis followed the standard protocol of the Association of Official Analytical Chemists (AOAC 1995). (1) Protein content was determined using Kjeldahl's method, and then the percentage of protein content (%) in each tube was calculated. (2) The fat content was determined using a dry sample treated with petroleum ether using the Soxhlet extraction method. (3) The fiber content was determined using dry samples, which were contained in Fibre Bag, and the fat was removed by immersion in acetone, then the content was determined using the Fibre Bag FIBRETHERM® – C. Gerhardt with a temperature of 105 °C ± 5 °C in a hot oven until the final constant weight was obtained. In addition (4) The sugar content of the sample was determined using the phenol–sulfuric acid method (Nielsen 2010).

Preparation of the mushroom extract

The mushrooms were extracted with water and ethanol solvents, the crude extracts were filtered and dried using an evaporator machine to obtain the dry extract samples, and the weight of each extract was obtained for the analysis of bioactive properties (Thongbai et al. 2013).

Alpha–Glucosidase inhibitory assay

100 µg of enzyme alpha–glucosidase was mixed at 0.35 units/ml with crude extracts of water and ethanol, then the crude extract in 10% DMSO was dissolved in 100mM phosphate buffer at pH 6.8 prepared at a concentration of 200 µg/ml with 50 µg of extract. The mixed solution was then incubated at 37 °C for 10 mins. After that, 4–nitrophenyl–α–D–glucopyranoside (pNPG) was added, at a concentration of 1.5 mM in 100 µg. The solution was incubated at 37 °C for 20 mins.,

and 1000 µg of 1M Na2CO3 was added to stop the reaction (Shai et al. 2011). Absorption was measured at 405 nm in the microplate reader. Finally, the percentage of alpha–glucosidase inhibition was calculated from the equation.

Statistical analysis

The mycelial growth rate was measured in different media, in various conditions including temperature, and pH. Furthermore, the spawn production of the mushroom strains was determined, and the data was statistically analyzed in terms of mean variance using the Tukey test with a significance of $P < 0.05$.

For the fruiting test trial, the fruiting bodies of three species of *Lentinus* sp. were harvested, counted, and weighed manually. Yield data and biological efficiency (B.E.) were recorded. Yield data was defined as the total weight of fresh mushroom per kilogram of the substrate (Royse 2010, Llarena–Hernández et al. 2011, Thongklang et al. 2014), biological efficiency (B.E.) means the weight of harvest/weight of dry substrate) × 100% (Razak et al. 2013, Liang et al. 2019, Thongklang et al. 2020).

Results

Phylogenetic analyses

The best RaxML tree with a final likelihood value of –7159.359546 is presented. The matrix had 534 distinct alignment patterns, with 35.74% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.246235, C = 0.223250, G = 0.274556, T = 0.255959; substitution rates AC = 0.774055, AG = 1.910722, AT = 1.219030, CG = 0.832219, CT = 3.625429, GT = 1.000000; gamma distribution shape parameter α = 0.176098. The Maximum parsimony tree scores for the consistency index (CI) = 0.669, retention index (RI) = 0.897, rescaled consistency index (RC) = 0.598, and homoplasy index (HI) = 0.333, length = 727. The result of BI analysis is accordant to ML and MP phylogenetic analysis, and ML was selected as the representative results.

Fig. 1 shows the ML phylogenetic tree of *Lentinus* species based on multi–gene DNA data set of ITS, LSU and RPB2 gene regions. The alignment comprised 78 collections (Table 1), with 3084 total characters including the gaps. Seven distinct clades were identified, which are sect. *Dicholamellatae*, sect. *Lentinus*, sect. *Lentodiellum*; sect. *Rigidi*, sect. *Tigrini*, *Poly–porellus* clade *I*, and *Poreporells* clade II. These results are consistent with the previous findings of Seelan et al. (2015). However, the two clades of *Polyporells* were surprisingly divided, a result which seems to disagree with previously published results. This unexpected division of *Polyporellus* should be examined in further studies (Fig. 1).

It was found that Thai species were distributed in three clades or sections. Firstly, sect. *Dicholamellatae,* comprised specimens of *L. badius* from tropical countries (Table 1), including a Thai specimen, and this clade was found to be related to a clade of *Poryporellus* II. Secondly, clade *Poryporellus* II comprised three species with elongated pores, which are *L. arcularius, L. brumalis* and *L. ciliates*; and two Thai specimens are included in the subclade of *L. arcularius.* The other three species, *L. polychrous, L. sajor–caju* and *L. squarrosulus*, are distributed in sect. *Rigidi,* and they were found to be identical to those species (Fig. 1).

Taxonomy

Lentinus arcularius (Batsch) Zmitr., International Journal of Medicinal Mushrooms (Redding) 12(1): 88 (2010) Figs 2–3

Index Fungorum number: IF543135; Facesoffungi number: FoF 14090

Pileus 10–40 mm diam., convex to applanate, deeply depressed at center, smooth, slightly glabrous, brown (6E4–6) when young, becoming light brown (6D4–6) when mature; margin attached with long spine–like, 2–5 mm long, fragile when mature, white to pale arrange (5A–3).

Pore decurrent, white when young, becoming orange–white to pale orange (5A2–3) with age, often rounded, mostly angular to radially elongated, 1–2 mm long, oblong at stipe zone, 3–4 mm long, with white eroded margin. Stipe central, 2–4 × 15–50 mm, cylindrical, smooth, white to concolorous with pileus with age, sometimes darker with dark brown (6F5–8), occasionally with white hyphae at base zone. Annulus absent. Context white at pileus and stipe, fleshy–tough to leathery. Oder and Taste not observed. Spore print white.

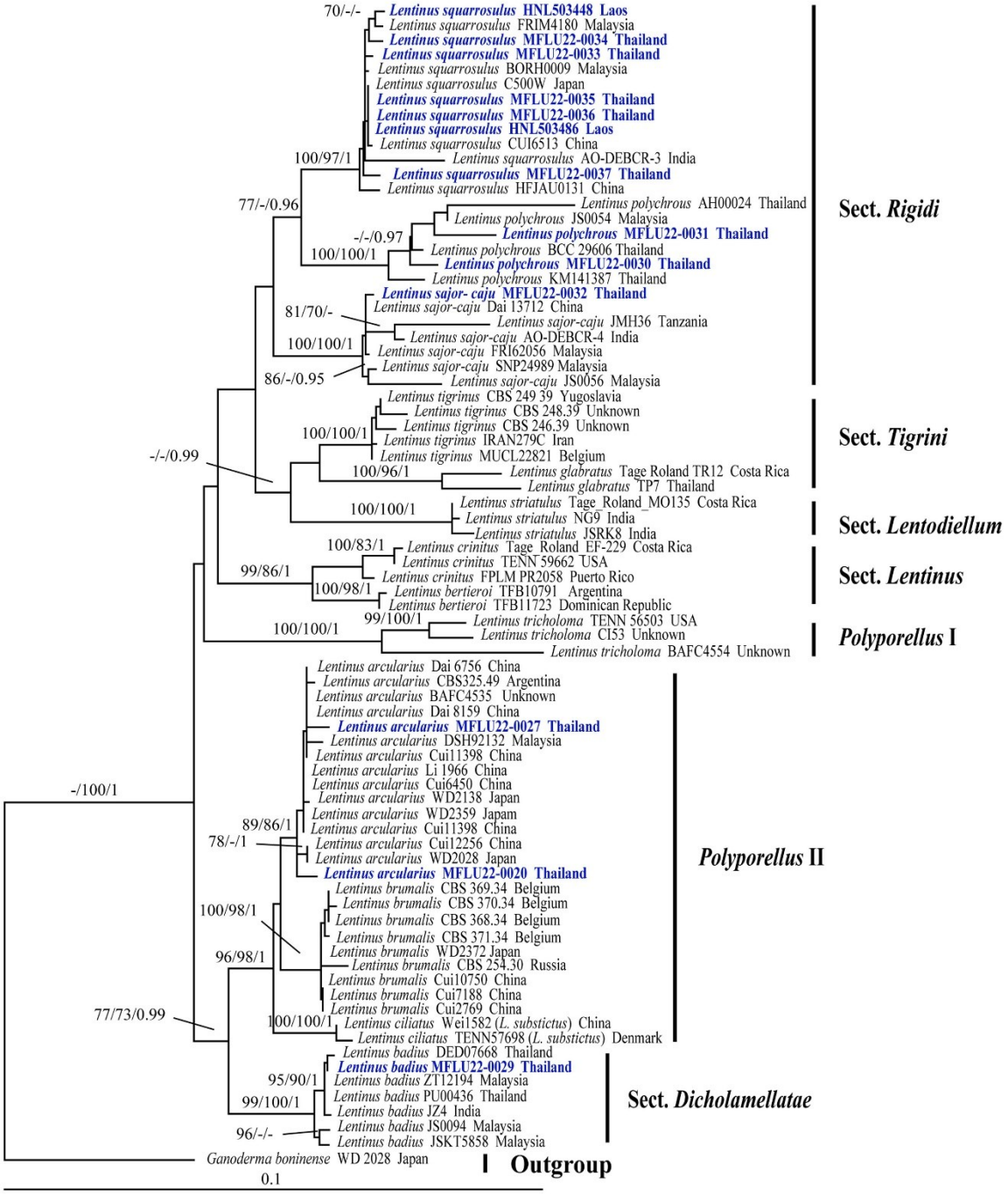

Fig. 1 – Maximum likelihood tree of *Lentinus* based on ITS–LSU sequences. The taxa in blue are specimens from Thailand. Support values of ML and MP≥70% and BI ≥ 0.95 are indicated on the nodes. Specimen voucher numbers are indicated after species names. *Ganoderma boninense* Pat. was used as the outgroup.

Hyphal system at tramal structure dimitic with generative hyphae and skeletal–ligative hyphae; generative hyphae thin–walled, hyaline, with clamp connection, 3–13 µm wide, cylindrical

to oblong with wider at middle zone; skeletal–ligative hyphae thick–walled, hyaline, up to 7–14 µm wide, thick–walled, with 2–5 lateral branches. Hyphae of long spine at marginal zone of pileus clustered with cylindrical elements with rounded or attenuate apex, slightly thick–walled, hyaline, very long, 5–7 µm wide at base, 2–3.5 µm wide at apex zone. Basidiospores 6.5–10 × 3–3.5 µm, oblong to cylindrical, thin–walled, hyaline, non–dextrinoid. Basidia clavate to 15–20 × 4.5–6.5 µm, thin–walled, hyaline, 4–spored. Cheilocystidia 25–40 × 4.5–7 µm, cylindrical with rounded apex, with 2–3 branches at basal zone, hyaline, slightly thick–walled. Pleurocystidia absent.

Habitat and distribution in Thailand – solitary or in a cluster, on dead wood in several forest types from low to high elevations. High distribution in northern and southern Thailand by this study. It was reported to distribute in northern and northeastern Thailand as *Polyporus arcularius* (Batsch) Fr. by Chandrasrikul et al. (2011) and Hjortstam & Ryvarden (1982).

Material examined – Thailand; Chiang Mai Province, Mueng District, Hua Doi Village, 13 July 2018, P. Sysouphanthong, PS2018–40 (MFLU22–0020); Chiang Rai Province, Muang District, Khun Korn Village, 27 August 2018, P. Sysouphanthong, PS2018–131 (MFLU22–0022); Chiang Rai Province, Pha Ngae Village, Pa Daed District, 29 August 2018, P. Sysouphanthong, PS2018–134 (MFLU22–0023); *ibidem*, 29 August 2018, P. Sysouphanthong, PS2018–162 (MFLU22–0024); *ibidem*, 29 August 2018, P. Sysouphanthong, PS2018–163 (MFLU22–0025); *ibidem*, 29 August 2018, P. Sysouphanthong, PS2018–165 (MFLU22–0026); Nan Province, Muang District. Phu Xang, 11 September 2018, P. Sysouphanthong, PS2018–186 (MFLU22–0027); Chiang Mai Province, Mae Taeng District, Pha Deng Village, 7 August 2018, coll. P. Sysouphanthong, PS2018–95 (MFLU22–0021).

Fig. 2 – Macrocharacters of *Lentinus arcularius*. a MFLU22–0021. b MFLU22–0022. c MFLU22–0023. d MFLU22–0025. e–f MFLU22–0024. g MFLU22–0026. h MFLU22–0020. i MFLU22–0027.

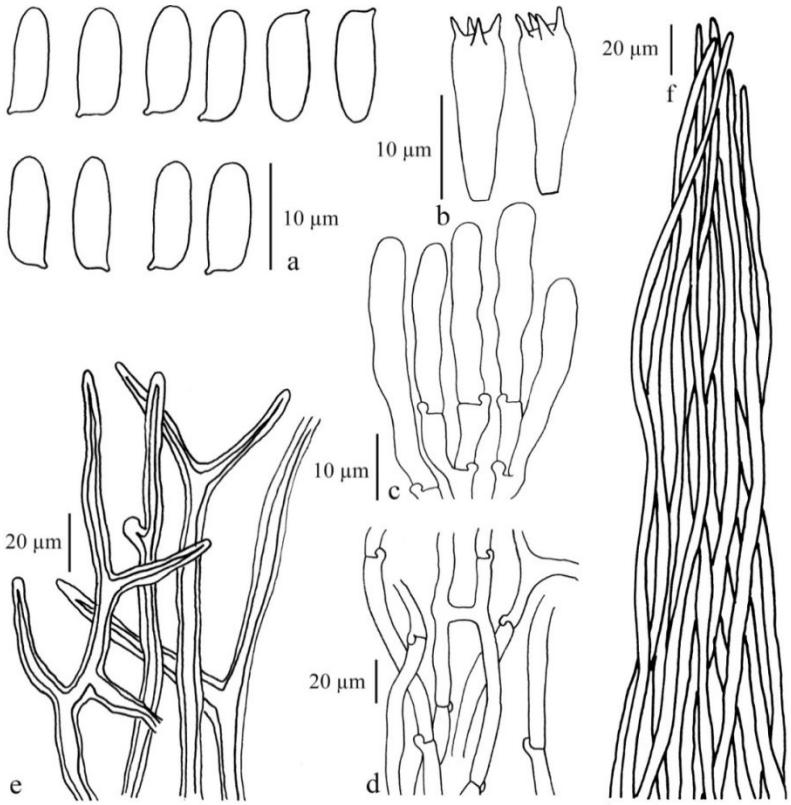

Fig. 3 – Microcharacters of *Lentinus arcularius* (MFLU22–0020). a basidiospores. b basidia. c cheilocystidia. d generative hyphae. e skeletal–ligative hyphae. f elements hyphae of long spine at marginal zone of pileus.

Notes – Thai collections have thin and various colours of pileus being light brown to brown, with short or long white spines at the marginal zones of the pileus, white to pale orange elongated pores, and central attachment and cylindrical stipe. Zmitrovich (2018) placed *L. arcularius* in *Lentinus* subgenus *Polyporellus* (P. Karst.) Zmitr., consisting of species with polyporoid basidiomata.

Thai specimens of *L. arcularius* in this study clustered well with those specimens from Argentina, China, India, Japan, Malaysia and Thailand (Table 1, Fig. 1).

Lentinus tricholoma (Mont.) Zmitr. appears similar to *L. arcularius* by morphology, but differs in having rounded or circular pores with smooth edges, while *L. arcularius* has elongated pores, and molecular data do not support that they belong to the same clade (Fig. 1) and is constant with Seelan eat al. (2015).

Lentinus brumalis (Pers.) Zmitr. is similar to *L. arcularius* by pileus colour and pore shape, but it has a thick pileus and short stipe, and absence of spines at marginal zone of pileus. Molecular data analysis in this study shows that they are in a clade with *Polyporellus* II that includes species with elongated to angular pores, and this is consistent with Seelan et al. (2015). *Lentinus brumalis* was recorded in northern Thailand by Hjortstam & Ryvarden (1982), but we do not find it in this study. *Lentinus substrictus* (Bolton) Zmitr. & Kovalenko has much larger basidiomata, greyish brown colour of pileus, absence of spine at pileus margin, and greyish brown squamules on a thick stipe (Zmitrovich & Kovalenko 2016).

Lentinus badius (Berk.) Berk., London J. Bot. 6: 491 bis (1847) Figs 4–5

Index Fungorum number: IF473358; Facesoffungi number: FoF 14089

Pileus 20–33 mm diam., convex when young with slightly depressed center, expanding to plano–convex to infundibuliform with depressed center, with inflexed to involved margin; background viscid and shiny when wet, smooth or glabrous when dried, with long striate margin,

8–10 mm long, greyish brown to brown (7E3–5) when young, lighter when mature, brownish orange to brown (6C4–6, 6D6–7), dark brown at center (6F7–8), covered with light brown to brown (6D4–6, 6E6–7) large warts at center toward margin; with white to light brown (6D4–6) warts at margin. Lamellae subdecurrent, white to orange–white (5A2–3), turned to greyish orange (5B3–4) with age, ventricose and wider at middle, smooth edge, furcate, with 2 branched levels, glutinous and solid when dry. Stipe central, 22 × 4–5 mm, cylindrical, with white fibrillose background, turned light brown (6D4–6) with age, covered concolorous warts same as pileus. Annulus with, membranous with split margin, attached at base zone of lamellae attachment. Context white at pileus and stipe, fleshy–tough to leathery, turned brown and solid when dried. Oder and Taste not observed. Spore print white.

Hyphal system at tramal structure dimitic with generative hyphae and skeletal–ligative hyphae; generative hyphae thick–walled, hyaline, with clamp connection, 4–6 µm wide, cylindrical to irregular cylindrical; skeletal–ligative hyphae thick–walled, hyaline, up to 8 µm wide, very long with 3–6 branches. Hyphae of scale on pileus irregular epithelium to hymenoderm made up of arranged elongate, short clavate, clavate, sub–clavate, sub–globose, globose elements, 13–25 × 5–15 µm, slightly thick–walled, pale brown, branched. Basidiospores 5.5–6.8 × 2.8–3.2 µm, smooth, oblong to cylindrical, slightly thin–walled, hyaline, non–dextrinoid. Basidia clavate, 20–25 × 5–7.5 µm, thin–walled, hyaline, 4–spored. Cheilocystidia absent. Pleurocystidia abundant, clustered, composed of several cylindrical to nettle hair–shaped, 50–90 × 3–10 µm, mostly wider or swollen at base zone (up to 12 µm wide) and tick–walled, cylindrical, or attenuate to apex (3–4 µm wide) and thin–walled, hyaline.

Habitat and distribution – Solitary, grows on dead wood, in wet rain forests with large trees and wet forest ground.

Material examined – Thailand; Krabi Province, Ao Luek District, Khlong Ya, 09 January 2020, P. Sysouphanthong, PS2020–20 (MFLU22–0029); Chiang Mai Province, Mae Kam Pong, 23 August 2019. P. Sysouphanthong, PS2019–85 (MFLU22–0028).

Fig. 4 – Macrocharacters of *Lentinus badius*. a–d MFLU22–0029 (mature). e–f MFLU22–0028 (young).

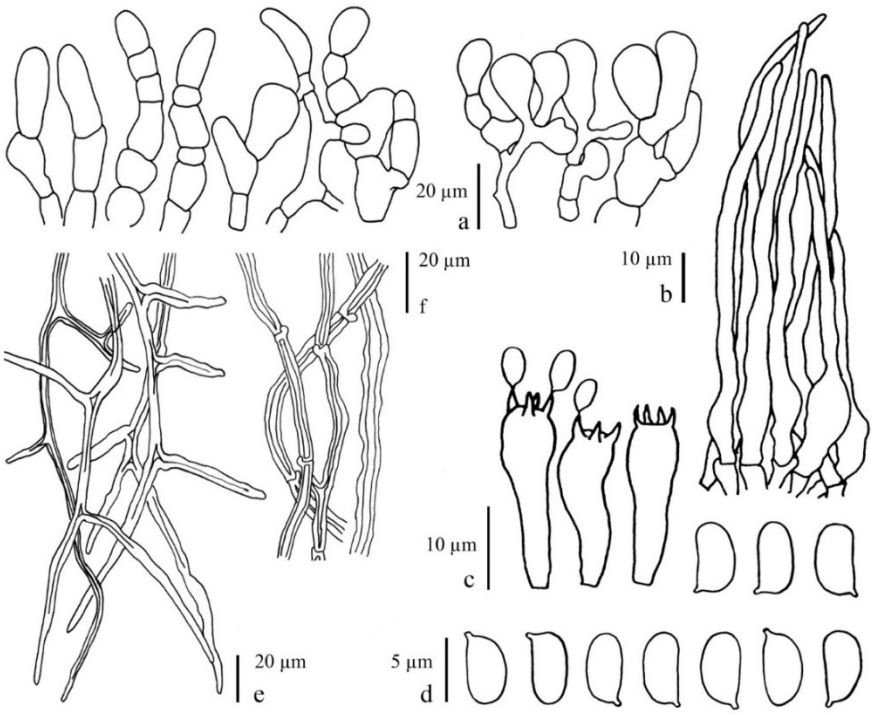

Fig. 5 – Microcharacters of *Lentinus badius* (MFLU22–0029). a element of scale on pileus. b a cluster of pleurocystidia. c basidia with young basidiospores. d mature basidiospores. e skeletal–ligative hyphae. f generative hyphae.

Notes – During our mushroom surveys of in Thailand, a single fruiting body was found in two collections was found; MFLU22–0028 was collected from Chiang Mai, northern Thailand, in a young stage without basidiospore production; and MFLU22–0029 was collected from Krabi, south Thailand, in a mature stage, from which the main description and ITS sequences were received from this species. However, *Lentinus badius* was reported from a dense humid forest in northern Thailand with abundant fruit bodies on rotten wood by Høiland & Schumacher (1982). Seelan et al. (2015) reported *L. badius* from central (Nakhon Ratchasima) and southern (Phuket) Thailand.

Lentinus badius is the only species in the sect. *Dicholamellatae* Pegler (Seelan et al. 2015), and was described from Sri Lanka by Berkeley (1847) based on macromorphology only, and a comprehensive description was absent in later studies. Available molecular data of *L. badius* are from tropical countries such as India, Indonesia, Malaysia, and Thailand (Seelan et al. 2015). A specimen of *L. badius* from this study clustered well with samples from those tropical countries (Fig. 1). *Lentinus badius* was also recorded from Brazil with smaller basidiospores (3.75–4.25 × 1.75–2 µm) (Baltazar & Gibertoni 2009, Drechsler–Santos et al. 2008).

According to available literature, *Lentinus badius* was only recorded in a checklist, and described based on macro–characters in Thailand. Here, we redescribe *L. badius* with morphology and molecular data, and its distribution in Thailand.

Lentinus polychrous Lév., Annls Sci. Nat., Bot., sér. 3 2: 175 (1844) Figs 6–7

Index Fungorum number: IF197977; Facesoffungi number: FoF 02273

Pileus 30–70 mm diam., convex to applanate, deeply depressed at the center, with concentrically fibrillose squamules at the center toward margin, yellowish brown (5E4–8) to brown (6E6–8), with yellowish white to pale yellow (4A2–3) background; margin split when mature, with concolorous fibrillose squamules as in pileus. Lamellae decurrent, yellowish brown (5E4–8) to brown (6E6–8), narrowed, 1–2 mm wide, with smooth margin. Stipe central to lateral, 4–7× 10–20 mm, equal with crowded fibrillose, concolorous with pileus. Annnuls absent. Context white at pileus and stipe, fleshy–tough to leathery. Odor and taste not observed. Spore print white.

Hyphal system at tramal structure dimitic with generative hyphae and skeletal–ligative hyphae; generative hyphae thick–walled, hyaline, with clamp connection, up to 9 µm wide, long cylindrical with wider near apex zone, with attenuate apex, up to 20 µm wide; skeletal hyphae–ligative hyphae ticked–walled, hyaline, up to 15 µm wide, very long and with 2–3 lateral branches, attenuate to apex. Hyphae at pileus not observed. Basidiospores 6.5–8.5 × 3–3.5 µm, oblong to cylindrical, thin–walled, hyaline, non–dextrinoid. Basidia 20–25 × 4–7 µm, long clavate with very narrow to base, thin–walled, hyaline, 4–spored. Cheilocystidia and pleurocystidia not observed.

Habitat and distribution – grow on dead wood in small groups with few to several fruit bodies. It is mostly found in dry dipterocarp forests in all parts of Thailand (Chandrasrikul et al. 2011).

Material examined – Thailand; Chiang Rai Province, Muang District, Mae Khao Tom, 12 July 2018, P. Sysouphanthong, PS2018–48 (MFLU22–0030); Chiang Rai Province, Muang District, Mae Khao Tom, 12 July 2018, Ladtana Keokaenngeun, LK44 (MFLU22–0031).

Notes – *Lentinus polychrous* is recognized by pale yellow to brown fibrillose to fibrillose squamules on pileus, yellowish brown to brown lamellae, central to lateral stipe attachment, absence of annulus. Two specimens in this study are identical to those specimens from Malaysia and Thailand (Fig. 1).

In Thailand, *Lentinus polychrous* was reported in all parts of Thailand as *L. praerigidus* Berk (Chandrasrikul et al. 2011, Phanichapol 1968, Høiland & Schumecher 1982, Pukahuta et al. 2008). The species is generally cultivated and sold in markets (Thawthong et al. 2014).

Lentinus polychrous is a tropical species, and it was originally described from Sumatra Island, Indonesia (Léveillé 1844). It was also found in India (Natarajan & Manjula 1978), Laos (Lee et al. 2021), Malaysia (Bolhassan et al. 2012), Philippines (Graff 1992), and Thailand as *L. praerigidus* Berk (Chandrasrikul et al. 2011, Phanichapol 1968, Pukahuta et al. 2008). This species is mostly distributed in South– and Southeast Asia, and it might exist in tropical areas of Africa and South America with other names, but literature is currently not available.

Fig. 6 – Macrocharacters of *Lentinus polychrous.* a–b MFLU22–0030. c–d MFLU22–0031.

Lentinus sajor–caju (Fr.) Fr., Epicrisis Systematis Mycologici: 393 (1838) Figs 8–9

Index Fungorum number: IF197304; Facesoffungi number: FoF 3142

Pileus 40–80 mm diam., infundiliform with deeply depressed center, smooth or glabrous, slightly rough with age, white when young, becoming yellowish brown to light brown (5D5–8) with age; margin smooth to wavy and broken when mature. Lamellae decurrent, white to yellowish brown (5E4–8), narrowed, 1–2 mm wide, with white eroded margin. Stipe central to lateral, 5–10× 10–15 mm, equal or slightly tapering to base, smooth, white. Annnuls present, attached at apex

zone of stipe, membranous, white, fragile when mature. Context white at pileus and stipe, fleshy–tough to leathery. Oder and Taste not observed. Spore print white.

Hyphal system at tramal structure dimitic with generative hyphae and skeletal–ligative hyphae; generative hyphae thin–walled, hyaline to pale yellow, with clamp connection, up to 6 µm wide; Skeleto–ligative hyphae dominant, up to 10 µm wide, hyaline, thick–wall, with long skeletal elements and attenuate to apex, up to 420 µm long, 2–4 lateral branches. Hyphae on pileus not observed. Basidiospores 6.5–10 × 2–3 µm, oblong to cylindrical, thin–walled, hyaline, non–dextrinoid. Basidia 18–22 × 4–6 µm, clavate, thin–walled, hyaline, 4–spored. Cheilocystidia clavate to fusiform, utriform, with or without appendage.

Habitat and distribution – grow on dead wood in large clusters, distributed throughout all parts of Thailand (Chandrasrikul et al. 2011, Høiland & Schumecher 1982, Pukahuta et al. 2008).

Material examined – Thailand; Chiang Rai Province, Muang District, Pong Pha Bath Village, 27 June 2018, Kevin D. Hyde, PS2018–24 (MFLU22–0032).

Notes – *Lentinus sajor–caju* is the only currently identified species with an annulus which can be used as a unique characteristic to distinguish it from other species. Tibpromma et al. (2017) noted that the species is widespread throughout Africa, Asia, and Australia. There are some available sequences of *Lentinus sajor–caju* from Thailand by Chukeatirote et al. (2012) and Grand et al. (2011). A specimen from this study clustered well with specimens from China, India, Malaysia, and Tanzania (Fig. 1).

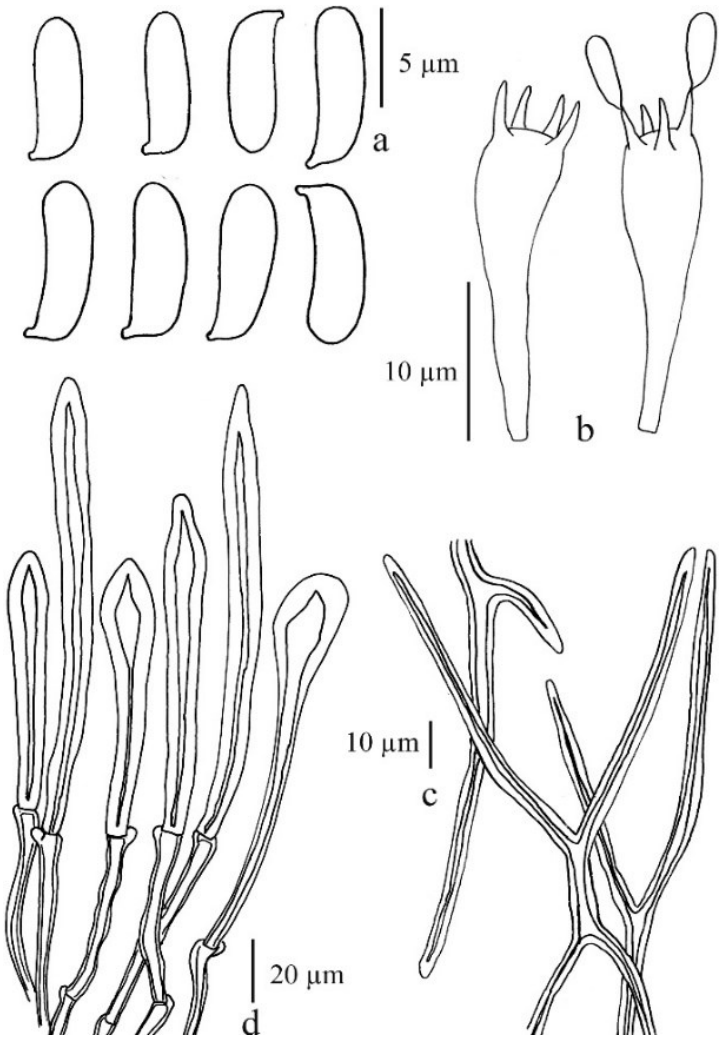

Fig. 7 – Microcharacters of *Lentinus polychrous* (MFLU22–0030). a basidiospores. b basidia. c skeletal–ligative hyphae. d generative hyphae.

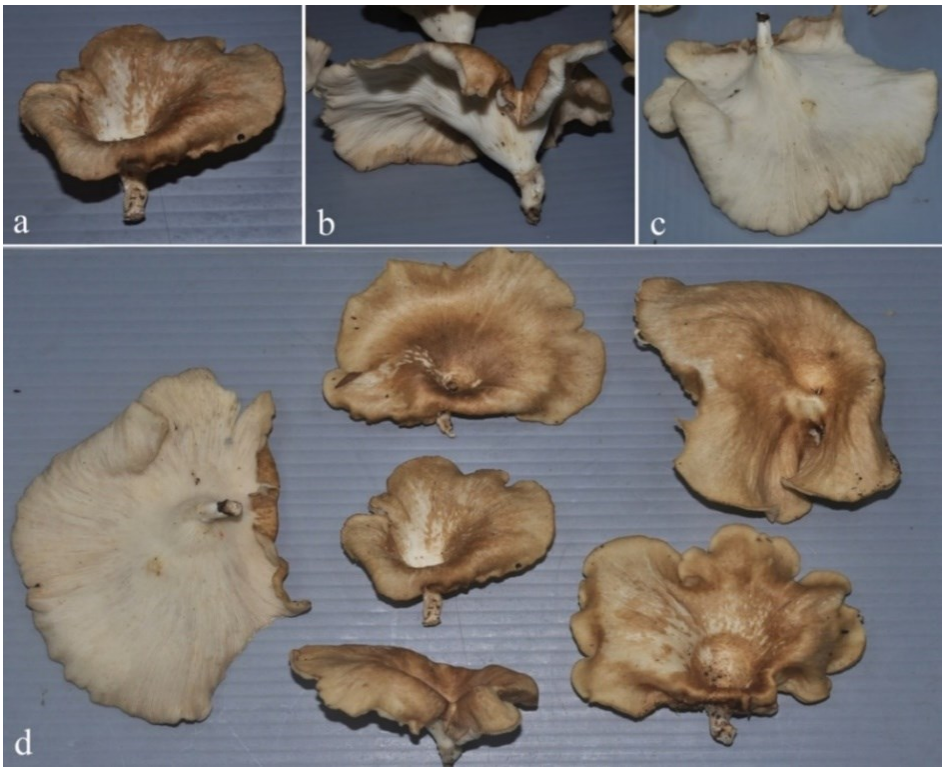

Fig. 8 – Macrocharacters of *Lentinus sajor–caju.* a–d MFLU22–0032.

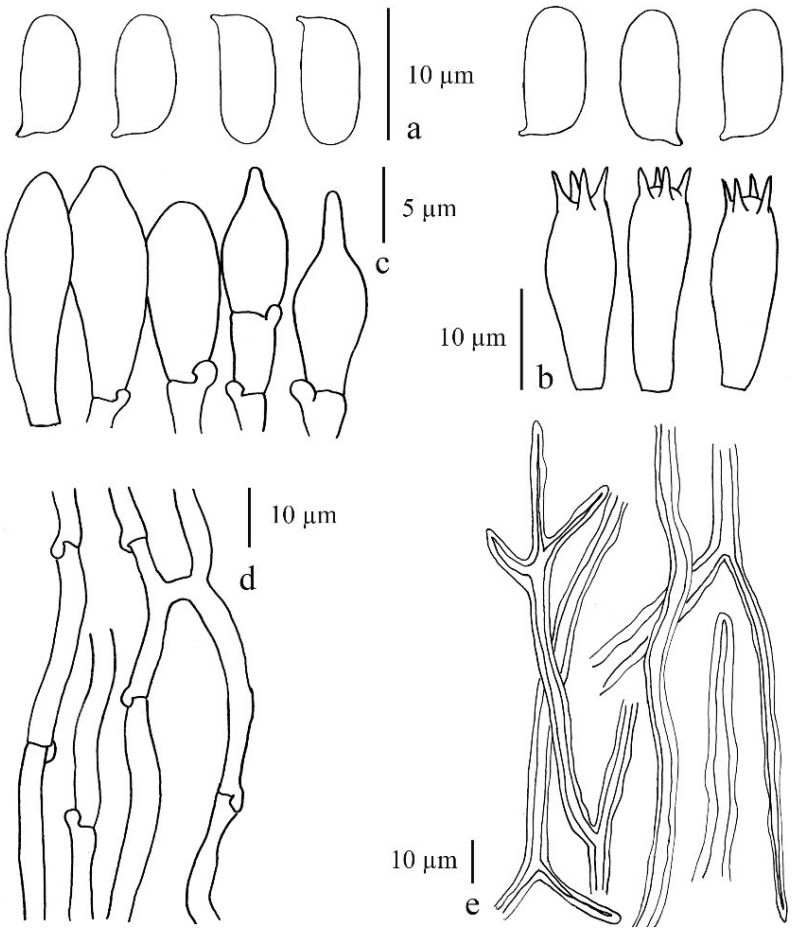

Fig. 9 – Microcharacters of *Lentinus sajor–caju* (MFLU22–0032). a basidiospores. b basidia. c cheilocystidia. d generative hyphae. e skeletal–ligative hyphae.

Lentinus squarrosulus Mont., Annales des Sciences Naturelles Botanique 18: 21 (1842)

Figs 10–11

Index Fungorum number: IF477648; Facesoffungi number: FoF 3143

Pileus 20–70 mm diam., convex to applanate with a depressed ~~at~~ center or infundiliform shaped, with concentrically fibrillose squamules crowded at center toward margin, yellowish brown (5E4–8) to brown (6E6–8), with white background, margin smooth or broken when mature, with white fibrillose. Lamellae decurrent, white, narrowed, up to 2 mm wide, with white smooth margin. Stipe central to lateral, 3–70× 20–40 mm, equal, smooth or with white fibrillose. Annulus absent. Context white at pileus and stipe, fleshy–tough to leathery. Oder and Taste not observed. Spore print white.

Hyphal system at tramal structure dimitic with generative hyphae and skeletal–ligative hyphae; generative hyphae thin–walled, hyaline, with clamp connection, up to 7 µm wide, branched; Skeletal–ligative hyphae up to 12µm wide, hyaline, thick–wall, with a continuous lumen, with 2–3 lateral branches. Hyphae on pileus not observed. Basidiospores 5–8 × 1.5–3 µm, cylindrical, thin–walled, hyaline, non–dextrinoid. Basidia 18–23 × 4–7 µm, clavate, thin–walled, hyaline, 4–spored. Cheilocystidia not observed.

Habitat and distribution – mostly grow in large clusters, rarely solitary; widespread in every forest type and found in all parts of Thailand (Chandrasrikul et al. 2011).

Material examined – Thailand, Chiang Rai Province, Toeng District, 19 June 2018, P. Sysouphanthong, PS2018–17 (MFLU22–0033); Chiang Mai Province, Muang District, Forest around Chiang Mai University, 19 May 2018, L. Keokaenngeun PS2018–51 (MFLU22–0034); Chiang Rai Province, Muang District, Hua Doi Village, 04 September 2018, P. Sysouphanthong, PS2018–183 (MFLU22–0037); Chiang Rai Province, Pa Daed Disrict, Pha Ngae, 29 August 2018, P. Sysouphanthong, PS2018–164 (MFLU22–0035); Chiang Rai Province, Mae Fah Luang District, forest near Doi Tung , 31 August 2018, coll. P. Sysouphanthong, PS2018–177 (MFLU22–0036).

Fig. 10 – Fresh basidiomata of *Lentinus squarrosulus.* a MFLU22–0037. b MFLU22–0034. c–d MFLU22–0035. e MFLU22–0033. f MFLU22–0036.

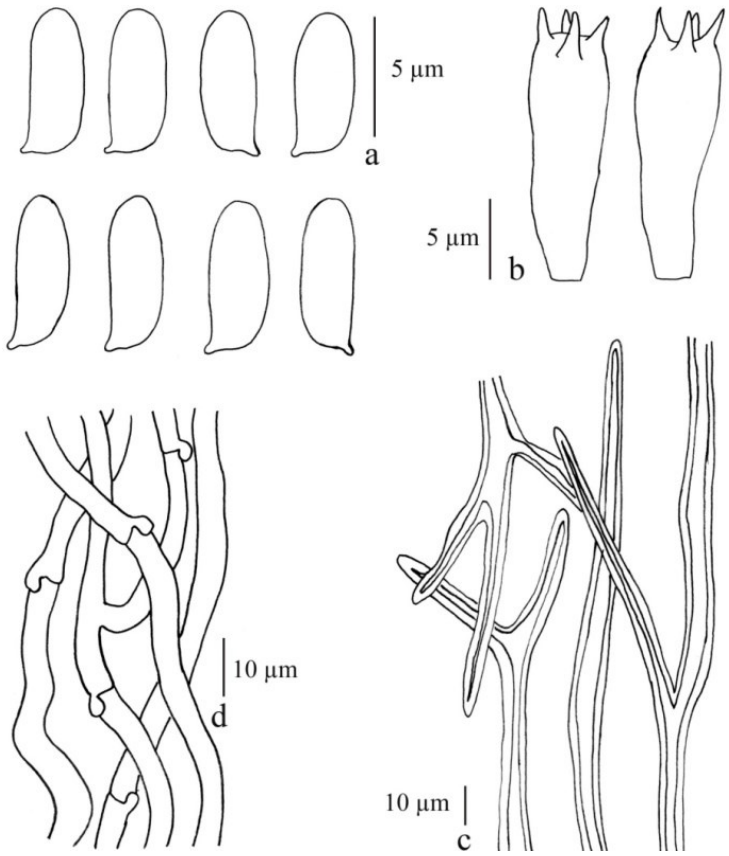

Fig. 11 – Microcharacters of *Lentinus squarrosulus* (MFLU22–0037). a basidiospores. b basidia. c Skeletal–ligative hyphae. d generative hyphae.

Notes – In this study, this species was very common and found in several different habitats such as grasslands, agricultural areas, degraded forests and evergreen forests (Chandrasrikul et al. 2011). This species is also cultivated and sold in markets (Thawthong et al. 2014, Pukahuta et al. 2008, Sakaew et al. 2013).

Tests of cultivation, nutritional values and screening of biological activities of *Lentinus*

Effect of media for mycelium growth

Three strains of *Lentinus*, *L. arcularius* (MFLU22–0021), *L. sajor–caju* (MFLU22–0032) and *L. squarrosulus* (MFLU22–0033), were selected for testing the effect of media for mycelium growth in different types of media (PDA, PSA, MEA, OMA, CMA) for 14 days. It was found that *L. arcularius* grew best on OMA compared to other media in a statistically significant manner with $P < 0.5$, and the average dry weight was 0.2279 ± 0.0195 g. While the optimal media to cultivate *L. sajor–caju* and *L. squarrosulus* was PSA. The average dry weight of mycelium of *L. sajor–caju* and *L. squarrosulus* were 0.1759 ± 0.0222 g and 0.2475 ± 0.0145 g, respectively (Table 2).

Effect of temperature on mycelial production

Each strain was inoculated in the optimum medium to check the mycelial growth and it was found that the medium PSA is the best for *L. squarrosulus* and *L. sajor–caju*, while the medium OMA is for *L. arcularius*. It was found that *L. squarrosulus* and *L. sajor–caju* covered the petri plates in eight days, while *L. arcularius* took 12 days, indicating slower growth. For mycelial weight, *L. squarrosulus* grew well at 25 °C and 30 °C, and the average dry weight was 0.0499 ± 0.0069 g and 0.0636 ± 0.0172 g, respectively while, *L. sajor–caju* grew well at 30 °C, and average dry weight was 0.1459 ± 0.0341 g. In addition, *L. arcularius* grew well at 25 °C, and the average dry weight is 0.2281 ± 02340 g (Table 2).

Effect of pH on mycelial production

These strains were then tested for mycelial growth at different pH conditions. In this experiment, the best medium for each strain was used based on the optimum medium test, which was used in this experiment, which were PSB for *L. squarrosulus* and *L. sajor–caju*, and OMB for *L. arcularius* at a pH of 2, 4, 6, 7, 8 and 10. The result showed a trend of *L. squarrosulus* to grow best at a pH of 6 compared to the other pH levels, but there was no statistically significant difference ($P > 0.5$), *L. sajor–caju* grew best at a pH of 6–8, and *L. arcularius* grew best at a pH of 4–10 with $P < 0.5$ (Table 2).

Effect of spawn for mycelial production

This experiment aimed to identify the optimum cereal for supporting mycelial growth for spawn production. Three strains (*L. squarrosulus*, *L. sajor–caju*, and *L. arcularius*) from the previous experiment were also used in this part. It was found that *L. squarrosulus* and *L. sajor–caju* were found to grow best on SRS, with an average mycelial length of 8.92 ± 0.53 cm and 14.0 ± 0.65 cm, respectively; while *L. arcularius* grew optimally on SCW and SRH, a result which was statistically significant $P < 0.5$, with a mycelial length of 6.86 ± 0.63 cm and 6.66 ± 0.23 cm, respectively (see Table 2, Fig. 12).

Table 2 Effect of media, temperature, pH, and cereal/waste substrates on the growth of three strains of *Lentinus* species.

Factors		***L. arcularius***	***L. sajor–caju***	***L. squarrosulus***
		(MFLU22–0021)	**(MFLU22–0032)**	**(FLU22–0033)**
Medium	PDA	0.1337 ± 0.0267^{c}	0.1373 ± 0.0016^{c}	0.1177 ± 0.0094^{c}
	MEA	0.1591 ± 0.0140^{bc}	0.1539 ± 0.0042^{bc}	0.1274 ± 0.0132^{c}
	OMA	$\mathbf{0.2279 \pm 0.0195^{a*}}$	0.1646 ± 0.0060^{ab}	0.1686 ± 0.0191^{b}
	CMA	0.0936 ± 0.0037^{d}	0.1028 ± 0.0104^{d}	0.0717 ± 0.0100^{d}
	PSA	0.1759 ± 0.0038^{b}	$\mathbf{0.1759 \pm 0.0222^{a*}}$	$\mathbf{0.2475 \pm 0.0145^{a*}}$
Temperature	20	0.1543 ± 0.0370^{b}	0.0519 ± 0.0027^{c}	0.0156 ± 0.0029^{b}
(°C)	25	$\mathbf{0.2281 \pm 02340^{a*}}$	0.1125 ± 0.0005^{b}	$\mathbf{0.0499 \pm 0.0069^{a*}}$
	30	0.1363 ± 0.0092^{b}	$\mathbf{0.1459 \pm 0.0341^{a*}}$	$\mathbf{0.0636 \pm 0.0172^{a*}}$
	40	0.0571 ± 0.0028^{c}	0.0125 ± 0.0005^{d}	0.0123 ± 0.0049^{b}
pH	2	0.0088 ± 0.0016^{b}	0.0106 ± 0.0046^{d}	0.0080 ± 0.0009
	4	$\mathbf{0.0647 \pm 0.0242^{a*}}$	0.0172 ± 0.0095^{cd}	0.0455 ± 0.0461
	6	0.0194 ± 0.0110^{ab}	$\mathbf{0.0545 \pm 0.0101^{a*}}$	0.0648 ± 0.0393
	7	0.0329 ± 0.0116^{ab}	0.0416 ± 0.0222^{ab}	0.0227 ± 0.0051
	8	0.0553 ± 0.0015^{ab}	0.0458 ± 0.0027^{ab}	0.0477 ± 0.0473
	10	$\mathbf{0.0712 \pm 0.0606^{a*}}$	0.0331 ± 0.0045^{c}	0.0454 ± 0.0217
Cereal/waste	S	5.86 ± 0.59^{b}	10.92 ± 0.98^{b}	6.06 ± 0.52^{c}
substrates	SRS	6.22 ± 0.41^{ab}	12.14 ± 1.52^{b}	7.54 ± 0.63^{b}
average length (cm)	SRH	$\mathbf{6.66 \pm 0.23^{a*}}$	11.82 ± 0.97^{b}	8.06 ± 0.13^{b}
	SCW	$\mathbf{6.86 \pm 0.63^{a*}}$	$\mathbf{14.0 \pm 0.65^{a*}}$	$\mathbf{8.92 \pm 0.53^{a*}}$

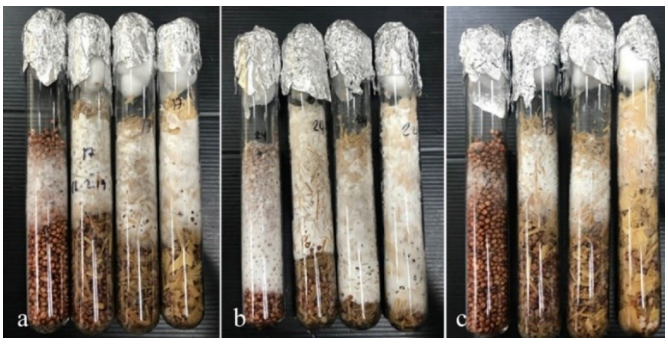

Fig. 12 – Mycelial of three strains grow on four substrate media at 25 ºC for 12 days. a *L. squarrosulus* (MFLU22–0033). b *L .sajor–caju* (FLU22–0032). c *L .arcularius* (MFLU22–0021).

Fruiting test of wild Thai *Lentinus* at laboratory scale

Wild strains of three species of *Lentinus*, two strains of *L. arcularius* (MFLU22–0023 and MFLU22–0026), one strain of *L. sajor–caju* (MFLU22–0032), and five strains of *L. squarrosulus* (MFLU22–0033, MFLU22–0034, MFLU22–0035, MFLU22–0034 and NTS 228 as a control) were used for the fruiting test.

Lentinus arcularius was isolated into PDA for 15 days and inoculated in sorghum grain medium. The spawn was inoculated into sawdust media bags (five replications) and incubated at 25 ± 2 °C in the dark. The mycelium fully colonized the substrate on day 66. Then the bags were incubated at 25–30 ± 1 °C and 70–80% of humidity. The primordia appeared on day 52.20 ± 1.10 for *L. arcularius* (MFLU22–0023) and on day 67.60 ± 0.55 for *L. arcularius* (MFLU22–0026). The average weight of *L. arcularius* (MFLU22–0023) was 34.00 ± 5.48 g. The yield data was 42.50 g/ kg^{-1} and biological efficiency (B.E.) was 50.90 ± .48%. The average weight of *L. arcularius* (MFLU22–0026) was 30.00 ± 7.07g. The yield data was 37.50 g/ kg^{-1} and biological efficiency (B.E.) was 44.91 ± 7.07% (Table 3). The cultivated *L. arcularius* is shown in Fig. 12.

The mycelium of *L. sajor–caju* (MFLU22–0032) fully colonized the substrate on day 39. Then, the primordia appeared on day 50–67, the average of the first primordia occurrence was on day 60 ± 7.09. The average weight of the mushroom was 20 ± 20 g. The yield data and biological efficiency (B.E.) were 25 g/ kg^{-1} and 29.94 ± 20.00% respectively (Table 3, Fig. 12).

For *L. squarrosulus*, it was found that mycelium of each strain took various amounts of time until their growth completely filled the bag as follows: MFLU22–0033 (39 days), MFLU22–0034 (28 days), MFLU22–0035, MFLU22–0037 (40 days) and the control strain NTS 228 (67 days); then each strain was moved to an open bag and incubated at a temperature of 25–30 ± 1 °C in 70–80% humidity. Three bags of strain MFLU22–0033 fruited at 57 days after opening, whereas the other two bags fruited at 61 and 114 days, and the average of the first primordial occurrence was 69 ± 25.10 days. The strain MFLU22–0034 produced the fruiting bodies 41–45 days after opening the bags, while the average of the first primordial occurrence was 43 ± 1.48 days. It took on average 52 ± 0.00 days for strain MFLU22–0035 to grow to completion in the bag assay. The strain MFLU22–0037 was 40–64 days, and the average day was 50 ± 10.04 days. The control strain NTS 228 produced the fruiting bodies 7 days after opening the bags. However, the yield of mushrooms was lower than the wild strains (MFLU22–0034, MFLU22–0035, and MFLU22–0037).

For yield production, strain MFLU22–0035 had the highest yield with the fastest growth 40 ± 10.00 g, yield data was 50.00 g/ kg^{-1}, and biological efficiency (B.E.) was 59.88 ± 10.00%. The result of the other strains are shown in (Table 3, Fig. 13).

Fig. 13 – The first cultivation of *Lentinus*. a–b *L. arcularius.* c–d *L. sajor–caju.* e–f *L. squarrosulus*.

Table 3 Comparison of the first flush yields of Thai *Lentinus* species.

***Lentinus* sp.**		**Primordia after inoculation (days)**	**Average weight (g/bag)**	**Yield data * (g/kg^{-1})**	**Biological efficiency (B.E.)**
L. arcularius	MFLU22–0023	52.20 ± 1.10	34.00 ± 5.48	42.50	50.90 ± 5.48
	MFLU22–0026	67.60 ± 0.55	30.00 ± 7.07	37.50	44.91 ± 7.07
L.sajor–caju	MFLU22–0032	60 ± 0.9	20 ± 20.00	25	29.94 ± 20.00
L. squarrosulus	MFLU22–0033	69 ± 25.10	21 ± 8.94	26.25	31.44 ± 8.94
	MFLU22–0034	43 ± 1.48	26.34 ± 15.39	32.93	39.43 ± 15.39
	MFLU22–0035	52 ± 0.00	40 ± 10.00	50.00	59.88 ± 10.00
	MFLU22–0037	50 ± 10.04	32 ± 10.95	40.00	47.90 ± 10.95
	NTF228	7.00 ± 0.00	26.00 ± 13.87	32.50	38.92 ± 13.87

Nutritional analysis of cultivated *Lentinus* species

A sample of *L. sajor–caju* and four samples of *L. squarrosulus* were extracted to measure nutritional values including protein, lipid, fiber, and carbohydrate content. It was found that 100 g of *L. sajor–caju* (MFLU22–0032) had 34.03 ± 0.30 g of fiber, 12.26 ± 0.07 of protein 6.14 ± 0.70 g of fat and 3.58 ± 0.67 g/100 g of carbohydrate.

It was found that 100 g of *L. squarrosulus* had around 12–17 g of protein, and the sample MFLU22–0037 had higher protein content (17.27 ± 0.16 g) than other samples. In addition, *L. squarrosulus* had 5–6 g of fat, 10–27 g of fiber, and 3–4 g of carbohydrates (Table 4).

Table 4 Nutritional value of wild strains of *Lentinus*.

Sample		**Nutritional value (g 100/g of sample)**			
		Protein	**Fat**	**Fiber**	**Carbohydrate**
L. sajor–caju	MFLU22–0032	12.26 ± 0.07	6.14 ± 0.70	34.03 ± 0.30	3.58 ± 0.67
L. squarrosulus	MFLU22–0033	14.03 ± 0.27	5.73 ± 0.14	27.52 ± 0.52	4.18 ± 0.15
	MFLU22–0034	12.56 ± 0.28	5.51 ± 0.53	16.31 ± 4.34	3.02 ± 0.16
	MFLU22–0035	16.79 ± 1.69	5.92 ± 1.05	14.83 ± 4.53	3.76 ± 0.33
	MFLU22–0037	17.27 ± 0.16	6.97 ± 0.23	10.12 ± 0.24	4.31 ± 0.13

Screening of biological activity of *Lentinus*

Alpha–Glucosidase inhibitory activity

It was found that the samples extracted with ethanol showed better inhibition of enzyme activity. The extracts from MFLU22–0032 (*L. sajor–caju*) and MFLU22–0037 (*L. squarrosulus*) with ethanol had an inhibitory effect on the enzyme alpha–glucosidase when compared with acarbose (Table 5).

Table 5 Inhibitory activity of alpha–glucosidase in wild Thai *Lentinus.*

Alpha–glucosidase	**% inhibition at 200 µg/ml**
Water extraction	
L. sajor–caju (MFLU22–0032)	10.26 ± 0.88
L. squarrosulus (MFLU22–0037)	inactive
Ethanol extraction	
L. sajor–caju (MFLU22–0032)	79.76 ± 0.37
L. squarrosulus (MFLU22–0037)	50.74 ± 0.61
Acarbose	62.05 ± 0.07

Discussion

Chandrasrikul et al. (2011) recorded 15 species of *Lentinus* in the checklist of mushrooms in Thailand; and Karunarathna et al. (2011) described three new species of *Lentinus* from northern

Thailand, which are *L. roseus*, *L. concentricus*, and *L. megacystidiatus*. However, those species have skeletal hyphae (without skeletal–ligative hyphae), and they should belong to the genus *Panus*. Based on morphological and molecular evidence, five species were found in this study, which are *L. arcularius, L. badius, L. polychrous, L. sajor–caju* and *L. squarrosulus*.

Molecular analysis showed that *Lentinus* is not a monophyletic genus. Seelan et al. (2015) conducted a study on *Lentinus* based on multiple genes analysis; they divided *Lentinus* into five sections and two *Polyporellus* clades; species in sect. *Dicholamellatae*, sect. *Lentinus*, sect. *Lentodiellum* and sect. *Rigidi* have lamellae; species in sect. *Tigrini* have subporoid lamellae; and species of two clades of *Poreporelles* have circular and angular pores. Later, Zmitrovich & Kovalenko (2016) and Zmitrovich (2018) divided *Lentinus* into two subgenera, which are *Lentinus* subgenus *Lentinus* Fr. and *Lentinus* subgenus *Polyporellus* (P. Karst.) Zmitr. However, the molecular phylogenetic analysis of this study was similar to that of Seelan et al. (2015), and we also identified and placed species into sections based on their study.

The combined evidence from taxonomic and phylogenetic studies is used in the selection of mushroom strains in culture experiments. Most of the commercial mushrooms in Thailand are those that have been imported from other countries and cultivated in Thailand. Two species of *Lentinus* are cultivated in Thailand, which are *L. squarrosulus* and *L. polychrous* (Thawthong et al. 2014). In this study, the cultivation of wild mushroom *L. squarrosulus*, and the first reported cultivation of wild mushrooms *L. arcularius* and *L. sajor–caju* were carried out.

Based on growth assays, the most suitable agar medium for *L. squarrosulus* and *L. sajor–caju* was potato sucrose agar (PSA) while for *L. arcularius* it was determined to be oatmeal agar (OMA). The mycelial growth on agar media of each *Lentinus* sp. significantly varied depending on the species and components of the media. There are many types of media that have been reported that can be used to grow the mycelium of *Lentinus*. *Lentinus squarrosulus* which also grew optimally on potato sucrose gelatin (PSG), and rice bran decoction gulaman (RBDG) (De Leon et al. 2017a, Kalaw et al. 2021). Coconut water gelatin (CWG) medium was suitable for *Lentinus sajor–caju* from the Philippines (De Leon et al. 2017b). While, wild Thai *Lentinus*, *L. conatus* and *L. roseus* mycelia grew well on black bean agar and red bean agar, respectively (Klomklung et al. 2014). The most suitable temperature for all three species of *Lentinus* (*L. arcularius*, *L. sajor–caju* and *L. squarrosulus*) ranged from 25 and 30 °C. The temperature at 30 °C was found to be suitable to grow many *Lentinus* species. (Klomklung et al. 2014, Dulay et al. 2021). The optimum pH for the culture of this *L. arcularius*, *L. sajor–caju,* and *L. squarrosulus* was at pH 4–10 while *L. conatus* and *L. roseus* favored a pH of 5–7 (Klomklung et al. 2014). Spawn production is one important procedure of mushroom cultivation. This step expands the mycelium of mushrooms before being cultivated in mushroom bags. In Thailand, sorghum grain is frequently used for spawn production. However, cereal/waste substrates can also be used. Coffee pulp, cotton waste, millet, sawdust, straw wheat can be used for spawn preparation (Phonemany et al. 2021). This study confirms that the best cereal/ waste substrates of those three species were corn waste + sorghum (1:1). w/w). In addition, rice husk + sorghum (1:1 w/w) is also suitable for *L. arcularius*, and it is better for spawn production than the sorghum alone.

In the laboratory–scale fruiting test, it was found that all wild mushrooms tested were able to be produce fruiting bodies in the laboratory. Two samples of *L. arcularius*, MFLU22–0023, and MFLU22–0026, were able to produce fruiting bodies easily and on the first attempt similar to *L. sajor–caju* (MFLU22–0032). This research is the first to report the cultivation of both of these wild mushrooms. Novel strains for mushroom cultivation experiments have also been reported in Thailand, such as *Pleurotus giganteus*, *Agaricus flocculosipes*, *Ag. subrufescens*, *Ag. subtilipes*, *Auricularia thailadica* and *Au. cornea* (Klomklung et al. 2012, Thongklang et al. 2014, 2016, 2020, Bandara et al. 2017). *Lentinus squarrosulus* is a novel/new strain cultured in Thailand. This mushroom can be purchased from markets in Thailand; however, it is not possible to identify the precise origin of the species that are cultivated and sold in the market. *Lentinus squarrosulus* is a popular mushroom eaten and cultivated in the northern and northeastern regions of Thailand. In this study, four samples of wild strains of *L. squarrosulus* (MFLU22–0033, MFLU22–0034, MFLU22–

0035, and MFLU22–0037) were compared with *L. squarrosulus* (NTF 228) that were purchased from the market. In the rubber sawdust bag cultivation, three strains out of four wild strains produced better yields than the commercial strain in the first flush. The rubber sawdust (*Hevea brasiliensis*) is commonly used as the main substrate to grow *Lentinus* sp. in Thailand. However, there are many economic tree species of sawdust that have been used as a main substrate of *Lentinus* cultivation for example *Brachystegia nigerica*, *Chlorophora excelsa*, *Celtis* sp., *Guera cedrata* and *Nesogordenia papaverifera* (Phonemany et al. 2021). In addition, it can be cultivated with other agricultural waste such as *Andropogon tectorum* straw, coffee parchment wastes, cotton waste, leaves and bark of fruit trees, maize cob, paddy straw, oil palm fruit fiber, rice husk, wood logs and wood sawdust (Chang et al. 1981, Fasidi & Kadiri 1993, Kadiri & Arzai 2004, Adesina et al. 2011, Chiejina & Osibe 2015, Roy Das et al. 2015, André–Ledoux et al. (2020). There is a potential use of local agricultural waste as an alternative substrate. Such recyclables can be used to reduce the cost of mushroom cultivation and can be promoted among farmers in the future.

Mushrooms have been considered a good source of nutritional value. They have been well–documented in terms of being an excellent source of protein, carbohydrates, minerals, and vitamins. However, the nutritional value of mushrooms varies depending on the species, substrate, and conditions that the mushrooms were cultivated (Phonemany et al. 2021). In this study, wild Thai *L. sajor–caju* had 34 g fiber per 100 g sample, and the *L. squarrosulus* had 10–27 g fiber per 100 g sample. While Nwanze et al. (2006) and Roy Das et al. (2015) reported that *L. squarrosulus* fiber contents were 7.64 and 8.32 g per 100 g samples. Moreover, there was a protein content of 12–17 g per 100 g sample, 5–6 g fat per 100 g sample, and 3–4 g carbohydrates per 100 g sample, suggesting that it can be a beneficial source of nutrition.

Mushrooms have nutritional value as well as medicinal properties. In this study, the screening of α–glucosidase inhibitory activity in Thai *L. sajor–caju* and *L. squarrosulus* was reported. It was found that extracts from mushrooms with ethanol could be used to measure such activity. These extracts had the effect of inhibiting the activity of the α–glucosidase enzyme when compared to standard Acarbose. Rungprom (2018) reported that the commercial *Lentinus* from a local farm in Thailand showed antihyperglycemic properties. The extracts of commercial *L. polychrous* and *L. squarrosulus* were determined to have alpha–glucosidase –glucosidase inhibitory activities. These preliminary results suggest that the two mushroom samples in this study could be further developed and studied for use as antidiabetic agents. However, further proximate, and biological activity studies are needed for wild Thai *L. arcularius*.

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