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New record of *Auricularia* in Thailand and optimization of different agricultural substrates for the cultivation of *Auricularia*

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Abstract

This study reports both the introduction of a new record and additional cultivation results for mushrooms of *Auricularia*. We have collected and established a new record of the strain MFLU24-0195 from the province of Chiang Mai, Thailand. Characterisation of this strain was based on morphological characters and DNA sequence data. Phylogenetic analyses indicate that the new strain clustered with *Auricularia fibrillfera* with a strong bootstrap, indicating the need for a new record to be introduced. For cultivation, two different *Auricularia cornea* strains and one strain of *A. fibrillfera* were tested on different agricultural substrates. The results showed that both *A. cornea* and *A. fibrillfera* grew well in sawdust + corn cob (*A. cornea* strain 1 (MFLUCC18-0346):290.87±17.62, *A cornea* strain 2 (MFLUCC18-0347): 280±16.54, and *A. fibrillfera* strain (MFLUCC24-0224): 265.97±17.45 g respectively). The first primordia were observed at 14±3.05, 15±3.21, and 15± 3.45 days, respectively. Our results therefore indicate that corn cobs can be considered for the cultivation of *Auricularia*.

Keywords – Agricultural wastes – Best Medium – Corn cob – Ear mushroom – New Record

Introduction

Auricularia Bull. is classified as a member of the family of Auriculariaceae in Basidiomycota. Wu et al. (2021) reported that this genus has 37 accepted species of *Auricularia* and *Auricularia mesenterica* (Dicks:Fr.) Pers is the type species (Wu et al. 2015). Mushrooms in this genus have several names including jelly fungi, ear mushrooms, and Hed-hoo-noo in Thai (Bandara et al. 2015). These mushrooms are commonly collected from tropical, subtropical, and temperate climates (Lowy 1952, Bandara et al. 2015, 2017). In Thailand, five species of *Auricularia* have been accurately identified (*A. asiatica, A. cornea, A. delicata, A.thailandica,* and *A. villosula*) based on molecular data and morphology (Bandara et al. 2017). It should be noted there have been seven further species previously

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reported, but the identifications were made based solely on morphology and most of these reports did not have a corresponding herbarium (Chalermpongse et al. 1998, Chamratpan 2003, Choieklin & Dhitaphichit 1999, Duengkae 2006, Petcharat et al. 1999, Sakolrak 2006). Regarding cultivation, commercially grown *Auricularia* mushrooms include, for example, *A. heimuer* F. Wu, B.K. Cui & Y.C. Dai and *A. polytricha* (Mont.) Sacc (Du et al. 2011, Wu et al. 2014a, b) which have been reported in China. Importantly, *Auricularia* spp. has been demonstrated to offer nutritional and therapeutic benefits (De Silva et al. 2012a, b) For example, *A. auricula-judae* (Bull.: Fr.) Queìl. has been shown to have antioxidant activity (Ukai et al. 1983, Yuan et al. 1998, Fan et al. 2006, Kho et al. 2009, Cai et al. 2015, Choi et al. 2018), and *A. polytricha* has been reported to have antibacterial, antihypercholesterolemic, and antioxidant properties (Sun et al. 2010, Zhao et al. 2015, Avci et al. 2016).

Auricularia mushroom is the fourth most cultivated mushroom genus after *Agaricus*, *Lentinula*, and *Pleurotus* (Chang 1996, Bandara et al. 2015) and is used as one of the most common ingredients in traditional Chinese medicine and cuisine (Yuan et al. 2019). It is popular for its taste, and has many health benefits, including immunomodulatory, antioxidant, anticoagulant, anticancer, and cholesterol-lowering qualities (Sękara et al. 2015). Interestingly, *A. fibrillifera* was originally described from Papua New Guinea (Kobayasi 1973) and it is very close to *A. thailandica* both in morphology and phylogeny (Wu et al. 2021). Both species are prevalent in Southeast Asia, and their morphology particularly the dry basidiomata makes it extremely difficult to distinguish between them. However, compared to *A. thailandica,* the fresh basidiomata of *A. fibrillifera* are softer (Wu et al. 2021). Considering the similarity and prevalence in the area to known species, it is noteworthy that, there has been no previous report of *A. fibrillifera* in Thailand.

The objective of this study was to cultivate *A. cornea and A. fibrillifera* using various agricultural wastes. For *Auricularia*, a growth was compared using rubber sawdust alone vs. rubber sawdust combined with various additives such as rice husk, rubber leaves, corncobs and straw. The findings of this study are examined, along with how these results may be relevant for industrial productivity. In addition, a new report on *A. fibrillifera* in Thailand is presented.

Materials & Methods

Fungal strains

Two previously described strains of *Auricularia cornea*, MFLUCC18-0346 (Thongklang et al. 2020) and MFLUCC18-0347 (Walker et al. 2023) were used for this study, which were collected from the province of Chiang Mai, Mae On District, was identified as *A. fibrillifera*. The fresh specimens were dried in hot air (40–50°C) and sealed in a Ziplock plastic bag. This strain was isolated by spore isolation subcultured in a PDA medium and incubated at 25°C for 14 days. The strain collection and dry specimen have been deposited in the Mae Fah Luang University Culture Collection (MFLUCC24-0224) and the Mae Fah Luang University Herbarium (MFLU24-0195).

Macro- and micromorphological character analyses

Morphological characteristics of *A. fibrillfera* were recorded. Macromorphological characters were described from fresh specimens. The photographs were taken *in situ* and in the laboratory. The colour notation of Kornerup & Wanscher (1978) was used. Micromorphological characters were obtained from free-hand sections of dried specimens. The tissues were mounted in H_2O and a 5% aqueous KOH solution and Congo red was used to highlight all structures.

DNA extraction, PCR, and sequencing

Dried basidiocarps of *A. fibrillfera* were used for molecular analysis. The sample was dried in desiccated conditions at 45°C and the DNA of the sample was extracted with the High Pure PCR Template Preparation Kit (Roche) following the manufacturer's protocol. DNA amplification was performed using primers for ribosomal DNA regions (ITS1/ ITS4) (White et al. 1990). The primer pairs LR5/LROR were used to amplify the region of nLSU following the PCR conditions described by Wu et al. (2015). Sequencing was performed by SolGent Co., Ltd, Yuseong-gu, Daejeon, South Korea. The sequence data was assembled using BioEdit v. 7.0.9.0 (Hall 1999) and subjected to a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the closest matches. The sequences of the Thai *A. fibrillifera* that were newly obtained for this study were deposited in GenBank (http://ncbi.nlm.nih.gov/genbank/submit/). Other sequences of this genus (Table 1) were obtained from Wu et al (2021).

Maximum likelihood analyses were performed in raxmlGUIv.0.9b2 (Silvestro & Michalak 2012) using the GTR + G model of evolution. Phylograms were visualized with the FigTree v1.4.0 program (Rambaut 2012) and in Adobe Illustrator CS5 (Version 15.0.0, Adobe, San Jose, CA).

Table 1 List of species, specimens and GenBank accession number of sequences used in this study.

Taxon name	**Herbarium code**	**GenBank accession number (ITS)**	**GenBank accession number (nLSU)**	**Country**
Auricularia americana	Dai 13636	KM396765	–	China
A. americana	Cui 11657	KT152095	KT152111	China
A. africana (T)	KM133591	NR177476	MZ669897	Uganda
A. africana	Ryvarden 44929	MH213349	MZ669897	Uganda
A. angiospermarum	Cui 12360	KT152097	KT152113	USA
A.angiospermarum (T)	BJFC 017274	NR151847	NG058579	USA
A. asiatica	BBH895	NR169914	–	Thailand
A. asiatica	Dai 16224	KX022011	KX022042	China
A. auricula-judae	Dai 13210	KM396769	KM396824	France
A. auricula-judae	MT 7	KM396771	KM396826	Czech Republic
A. australiana (T)	MEL 2385783	NR176760	NG088285	Australia
A. australiana	HT 190	MZ647503	MZ669920	Australia
A. brasiliana (T)	URM 85567	NR151845	KP729293	Brazil
A. brasiliana	BDNA 1641	KP729277	KP729295	Brazil
A. camposii	URM 83464	MH213352	MH213396	Brazil
A. camposii (T)	URM 76905	MH213351	MH213395	Brazil
A. conferta (T)	BJFC 027293	NR174873	NG079707	Australia
A. conferta	Dai 18825	MZ647500	MZ669901	Australia
A. cornea	YG-Dr1	MH213353	MH213397	Germany
A. cornea	Dai 12587	KX022012	KX022043	South Africa
A. cornea	Dai 15336	KX022014	KX022045	China
A. cornea	Wu 07	MH213354	MH213398	China
A. cornea	Dai 17352	MH213355	MH213399	Ghana
A. cornea	Lira 663	MH213359	MH213403	Brazil
A. cornea	MFLU1304	KX621145	–	Thailand

Table 1 Continued

Taxon name	Herbarium code	GenBank accession number (ITS)	GenBank accession number (nLSU)	Country
A. cornea	MFLU162104	KX621144	–	Thailand
A. cornea	MFLU19-0797	MK696312	–	Thailand
A. cornea	MFLU23-0259	OR105042	–	Thailand
A. cornea	MFLU18-0199	OR105024	–	Thailand
A. delicata (T)	P 14	MH213364	MZ669933	Cameroon
A. fibrillifera	Dai 13598A	KP765615	KP765629	China
A. fibrillifera (T)	F 234519	KP765610	KP765624	Papua New Guinea
A. fibrillifera	Cui 6318	KP765611	KP765625	China
A. fibrillifera	Dai 18486	MH213365	MZ669923	Zambia
A. fibrillifera	Cui 6704	KP765613	KP765627	China
A. fibrillifera	**MFLU24-0195**	**PP935774**	**PP935773**	**Thailand**
A. fuscosuccinea	FP102573SP	KX022027	KX022058	USA
A. fuscosuccinea	Dai 17406	MH213366	MH213407	Brazil
A. heimuer	Dai 13503	KM396789	KM396840	China
A. heimuer (T)	Dai 13765	KM396793	KM396844	China
A. lateralis (T)	Dai 15670	KX022022	KX022053	China
A. mesenterica	Haikonen 11208	KP729287	KP729305	United Kingdom
A. mesenterica	Miettinen 12680	KP729286	KP729304	Switzerland
A. minutissima(T)	Dai 14881	KT152104	KT152120	China
A. minutissima	Dai 14880	KT152103	KT152119	China
A.nigricans	Ahti 55718	MH213372	KM396850	Costa Rica
A. nigricans	TJY 93242	KM396803	KM396851	USA
A.novozealandica	PDD 88998	KX022035	KX022066	New Zealand
A.novozealandica (T)	PDD 83897	KX022034	KX022065	New Zealand
A. orientalis (T)	Dai 14875	KP729270	KP729288	China
A. orientalis	Dai 1831	KP729271	KP729289	China
A. Pilosa (T)	LWZ 20190421-7	MZ647506	–	Ethiopia
A. pusio	AK 547	MH213374	MH213414	Australia
A. pusio	Smith 18	MH213375	MZ669926	Zambia
A. scissa (T)	TFB 11193	JX065160	–	Dominican Republic
A. scissa	Ahti 49388	KM396805	KM396853	Dominican Republic
A. sinodelicata	Cui 8596	MH213376	MH213415	China
A. sinodelicata (T)	Dai 13926	MH213379	MZ669909	China
A. subglabra	Dai 17403	MH213382	MH213419	Brazil
A. srilankensis (T)	Dai 19522	MZ647501	MZ669912	Sri Lanka
A. srilankensis	Dai 19575	MZ647502	MZ669913	Sri Lanka
A. submesenterica (T)	Dai 15450	MH213386	MH213420	China
A. submesenterica	Dai 15451	MZ618942	MZ669914	China
A. thailandica (T)	MFLU 130396	KR336690	–	Thailand
A. thailandica	Dai 15080	KP765622	KP765636	China

Table 1 Continued

Taxon name	Herbarium code	GenBank accession number (ITS)	GenBank accession number (nLSU)	Country
A. thailandica	Dai 13655A	KP765619	KM396863	China
A. thailandica	Dai 15335	KX022026	KX022057	China
A. thailandica	Dai 13759	KP765621	KP765635	China
A. tibetica (T)	Cui 12267	KT152106	KT152122	China
A. tibetica	Dai 13636	KM396765	–	China
A. tibetica	Cui 12337	KT152108	KT152124	China
A. tremellosa	Dai 17415	MH213390	MH213424	Brazil
A. tremellosa	AJS 1304	JX065158	–	Mexico
A. villosula	LE 296422	NR137873	–	Russia
A. villosula	Hei 01973	MZ618944	MZ669916	China
Exidia qinghaiensis (T)	HMAS 156328	NR172805	–	China

Notes – Newly produced sequences are in **bold** and "T" indicates type species (T). Ahti: name of the voucher; AJS: name of the isolate; AK: name of the voucher; BBH:Biotec Bangkok Fungarium; BDNA: name of the voucher; BJFC: Beijing Forestry University; Cui: name of the voucher; Dai: name of the voucher; F: name of the voucher; FP: name of the isolate; Haikonen: name of the voucher; Hei: name of the voucher; HMAS: Institute of Plant and Microbial, Academia Sinica; HT: name of the voucher; KM: Mycology collection of Kew botanical garden; LE: Komarov Botanical Institute, Russia; Lira: name of the voucher; LWZ: name of the voucher; MEL: Royal Botanic Garden Victoria, Australia; Miettinen: name of the voucher; MFLU: Mae Fah Luang University, Thailand; MT: name of the voucher; Ryvarden: name of the voucher; P: name of the voucher; PDD: name of the isolate; Smith: name of the voucher; TFB: name of the isolate; TJY: name of the voucher; URM: Universidade Federal de Pernambuco, Brazil; Wu: name of the voucher; YG-Dr1: Yusufjon Gofforov-Dr1

Spawn production

For spawn production, *Sorghum bicolor* (sorghum) grains were used (Thongklang & Luangharn 2016). After being cleaned and soaked overnight, the grains were boiled for 15 minutes. Bottles containing 100 grams of grains were autoclaved at 121°C for 15 minutes before being allowed to cool. One-fourth of a PDA plate containing mushroom mycelium was used to inoculate the bottles. For 30 days, the inoculated bottles were incubated at 25°C in the dark.

Suitable agricultural wastes for cultivation

In this experiment, ten different formulae were tested for *each* strain of *Auricularia.* Rubber sawdust was used as the main substrate. Each substrate was mixed (w/w) and then supplemented with 5% of rice bran, 1% of spent brewery grain, 1% of glutinous rice flour, 1% of pumice sulfate, and 1% of calcium carbonate. All substrate supplements were manually mixed with 70% moisture. The mixture (600g) was packed into polypropylene bags and then capped with a plastic ring and lid. The sawdust bags were sterilised at 121°C for 45 minutes. After the temperature cooled to 25°C, 50 g of spawn were inoculated into sawdust bags under aseptic conditions. The bags were incubated at 25±3°C in the dark, for 60 days. For the fruiting phase, the same temperature and 75-85% humidity were used. The mushroom yield of each strain and the first primordia were recorded.

Results

Taxonomy

Auricularia fibrillifera Kobayasi, Bull. natn. Sci. Mus., Tokyo 16(4): 645 (1973) (Fig. 1)

Basidiocarp: with a width up to 5 cm, and up to 0.4 mm thick, stalk absent, attached to the substrate from the center, ear-shaped basidiocarp, soft and gelatinous, reddish brown (7B7). Smooth on the upper surface and with hymenophore folds.

Internal features: thickness 320–650 µm Medulla indistinctly present in the middle of the cross-section; crystals present, very small, scattered throughout the cross-section; swollen base of abhymian hair, hyaline, thick-walled, hyphae with clamp connections, 1.3–3.2 µm; basidia clavate, transversely 3-septate, with oil guttules, 43–60 × 4–6 µm, sterigmata rarely observed; No cystidioles. Zona pilosa 70 µm; zona compacta 4-6.5 µm; zona subcompacta superioris 10–12 µm; zona laxa superioris 100 µm; medulla 40–50 µm; zona laxa inferioris 120-150 µm; zona subcompacta inferioris 25 µm, abhymenial hairs 54–88 × 12–18 µm, Cylindrical shape, Basidiospores (10.7–)11–14(–14.5) ×4–5(–5.6) µm, Q = 1.7-2.3, Qav= 2.0., allantoid, hyaline, thin-walled, and smooth, usually with one or two large guttules.

Material examined: Thailand, Chiang Mai, Mae On District, 19 July 2023, Sabin S2 (MFLU24-0195).

Distributions: Africa, Asia and Oceania (Wu et al. 2021). This is the first record from Thailand.

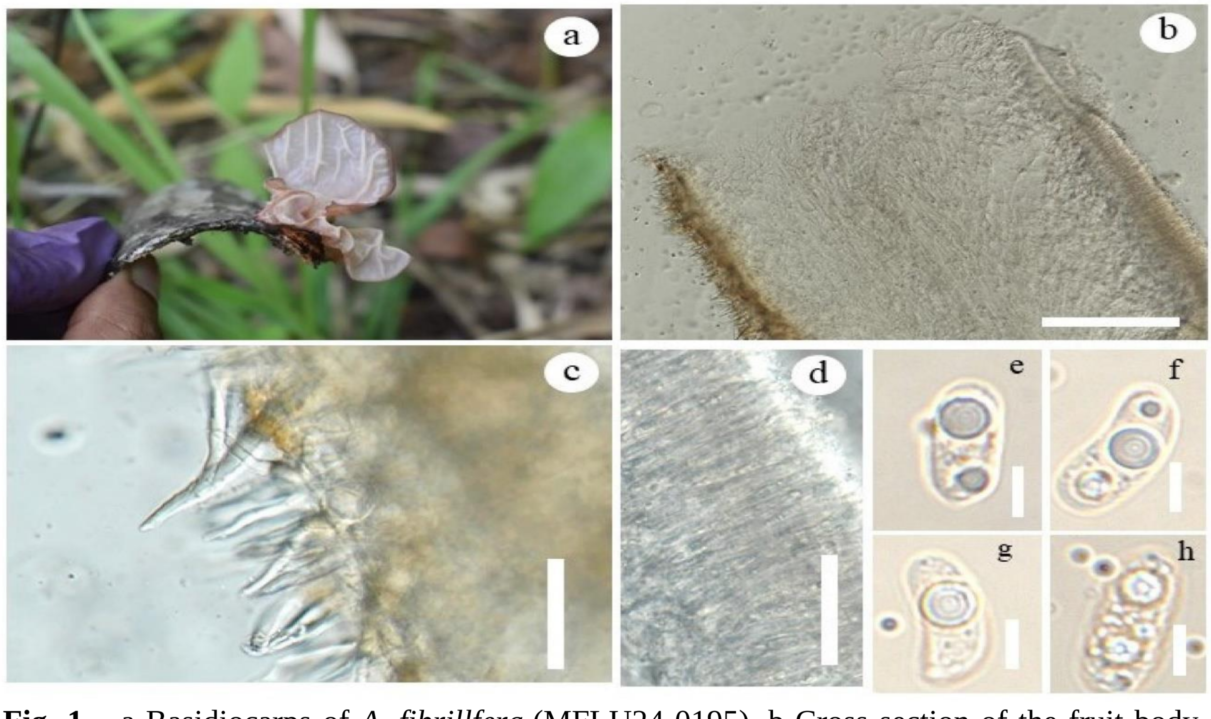

Fig. 1 – a Basidiocarps of *A. fibrillfera* (MFLU24-0195). b Cross section of the fruit body. c Abhymenial hairs. d Close-up of a hymenial layer. e−h Basidiospores. Scale bars: b = 500 µm, c = 50 µm, d = 25 µm, e−h = 5 µm.

Notes – In general, the key criteria used to distinguish *A. fibrillfera* were soft gelatinous Basidiomata present when fresh, hymenophore surface usually with folds; medulla indistinctly present, abhymenial hairs 60–100 × 10–20 µm. We discovered that the internal features of our *A. fibrillfera* differ slightly in regards to the sizes of abhymenium hairs from *A. fibrilfera* described (Wu et al. 2021). The abhymenium hairs of our *A. fibrilfera* were slightly smaller (54–88 × 12–18 µm). *A. fibrillifera* was originally described from Papua New Guinea (Kobayasi 1973), and the type specimen (F 234519) was previously reported. Interestingly, both the reports from Bandara et al. 2015 and Wu et al. 2021 pointed out that *Auricularia thailandica* Bandara & K.D. closely resemble *A. fibrillifera*. These two different species are similar in both morphology and phylogeny and have been found in Southeast Asia (Wu et al. 2021). The main differences were *A. fibrillfera* had thick-walled, acute, free or partly fasciculate hairs and thinner zones (Kobayasi, 1981, Bandara et al. 2015) and the fresh fruiting body of *A. fibrilfera* seemed softer than *A. thailandica* (Wu et al. 2021). In addition, these two species clustered into two distinct lineages in our phylogeny based on ITS+nLSU dataset (Fig. 1).

Phylogenetic Analyses

The final alignment of the ITS+nLSU dataset comprises 75 strains including the outgroups. The analysis of maximum likelihood showed that MFLU24-0195 clustered with the strains of *A. fibrillfera* with strong bootstrap support (Fig. 2). Our *A. fibrilfera* clustered in the clade with the type species (F 234519) with strong bootstrap support. The final matrix contained 157 distinct alignment patterns and the likelihood of the best scoring ML tree was −21,271.48146. The alpha scores: 0.176054, Tree-Length: 1.185975, rate A <-> C: 3.047241, rate A <-> G: 2.350963, rate A <-> T: 2.647193, rate C <-> G: 1.288824, rate C <-> T: 11.778712, rate G <-> T: 1.000000. The topologies obtained from the ML analysis were similar to previous studies. The ML analysis provided concrete evidence that MFLU24-0195 is *A. fribrillfera*, which is a new record for Thailand.

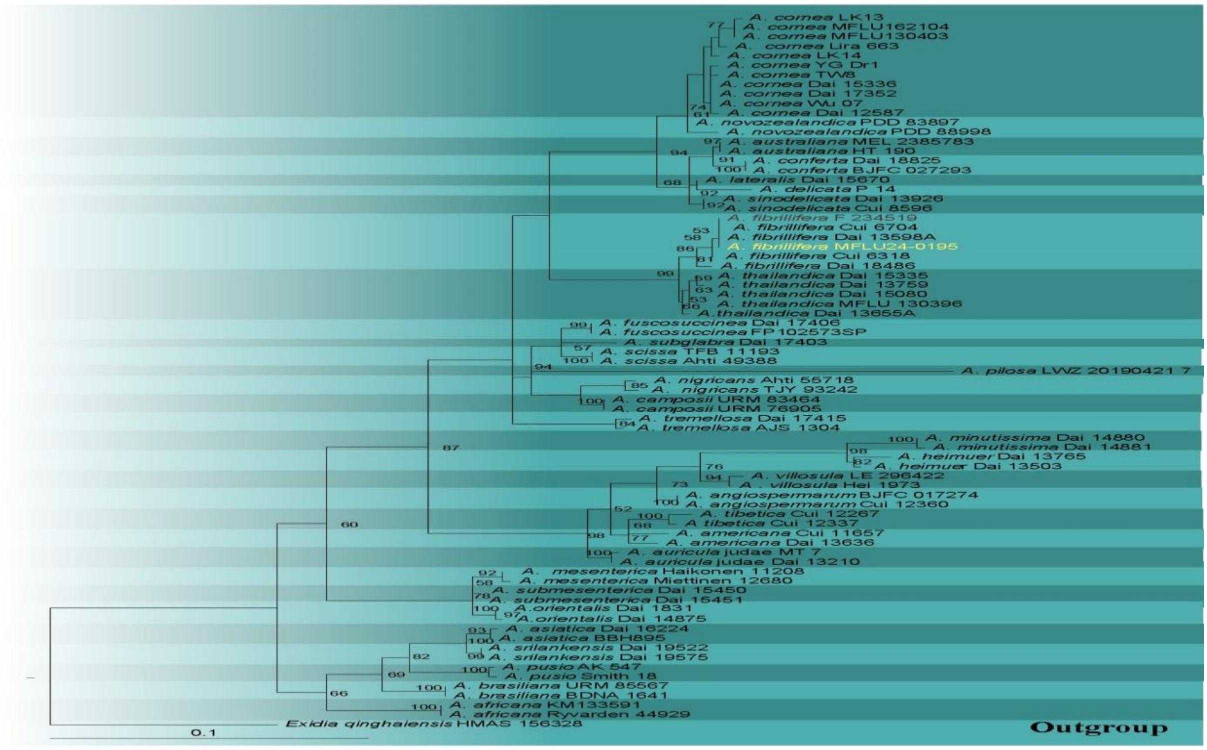

Fig. 2 – Maximum Likelihood (ML) tree illustrating the phylogeny of *Auricularia* based on ITS + nLSU dataset. The tree is rooted with *Exidia qinghaiensis*.

Optimal Bag Cultivation

Cultivation of three strains (MFLUCC18-0346, MFLUCC18-0347 and MFLUCC24-0224) of *Auricularia* was carried out with five replicates (Fig. 3). The first primordia were observed in the cultivation substrate that was a mix between sawdust and corn cob for all three strains (14±3.05, 15±3.21, and 15±3.45 days). The total yield of all flushes was 290.87±17.62, 280±16.54, and 265.97±17.45 g respectively (Table 2).

Fig. 3 – Cultivated basidiocarps on sawdust + corn cob of different *Auricularia*. a *Auricularia cornea* (MFLUCC18-0346). b *Auricularia cornea* (MFLUCC18-0347). c *Auricularia fibrillifera* (MFLUCC24-0224).

Discussion

Several reports mentioned that *Auricularia cornea* and *A. heimuer* are being grown for sale in China, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam and so can be considered commercially important (Chang & Lee 2004, Duc 2005, Tapingkae 2005, Peng 2008, Wu et al. 2014). More than 90% of the world's yield of *A. auricula* is currently produced in China. It is noteworthy that for *A. auricula* dry products, as much as 674,000 tons were produced in 2018 (Yu et al. 2023). Despite its potential importance, *A. fibrillfera* cultivation has been previously mentioned by only a single report (Zhang et al. 2019). Our study is the first to demonstrated that *A. fibrillfera* grows on a mixture of sawdust and corn cob.

Presenting a challenge in distinguishing *Auricularia fibrillfera* from other *Auricularia*, the sizes of the zona pilosa of *A. fibrillfera* are similar to *A. thailandica*, *A. fuscosuccinea*, *A. minor, A. scissa,* and *A. subglabra*. These species have been distinguished previously primarily due to *A. fibrillfera* having narrower zones and thicker walls with acute, free, or partially fasciculate hairs (Kobayasi 1981, Bandara et al. 2015); also, the fruiting body of *A. fibrillfera* appeared softer than *A. thailandica* (Wu et al. 2021). Furthermore, according to our phylogeny based on the ITS+nLSU dataset, *A. fibrillfera* clustered with different lineages of *A. thailandic.* Critically, MFLU24-0195 clustered with the type species (F 234519) providing strong evidence that MFLU24-0195 can be treated as *A. fibrillfera*, which is a new record in Thailand.

Compost and agro-waste products are used in many Southeast Asian nations for the low-cost cultivation of *Auricularia* species. For example, *A. auricula-judae* was successfully cultivated in India using compost that was predominantly made of corncobs, rice straw, broadleaf tree sawdust, and cottonseed bran with plaster stone, wheat bran, rice bran, and quick lime as supplemental components (Verma & Verma 2017). Our study found that both *A. cornea* and *A. fibrillfera* grew best on a mixture of corn cob and sawdust. Further, our results parallel previous research from Kenyan studies which reported that *A. auricula* cultivated on maize cobs and wheat bran were found to be optimal (Onyango et al. 2011). As has been previously described, we might have expected to see a greater difference between species if we had altered other additives (such as rice bran) (Onyango et al. 2011).

Table 2 Comparison of mushroom yield for each treatment

Treatment	*Auricularia cornea*						*Auricularia fibrillifera*		
	MFLUCC18-0346			MFLUCC18-0347			MFLUCC24-0224		
	First Primodia (Day)	**No. of flushes**	**The total yield of every flush (5 replicates)**	**First Primodia (Day)**	**No. of flushes**	**The total yield of every flush (5 replicates)**	**First Primodia (Day)**	**No. of flushes**	**The total yield of every flush (5 replicates)**
1 (SD+ RS)	28±3.14^{d}	4	117.82±11.11^{e}	26±2.22^{c}	4	98.21±12.21^{gh}	25±2.22^{c}	5	100.34±10.93^{g}
2 (SD+ RH)	16±2.05^{ab}	6	127.5±13.29^{de}	17±2.19^{ab}	6	122±12.45^{f}	15±2.98^{a}	6	131.11±14.67^{f}
3 (SD + Corn)	14±3.05^{a}	6	290.87±17.62^{a}	15±3.21^{a}	6	280±16.54^{a}	15±3.45^{a}	6	265.97±17.45^{a}
4 (SD+ Rb)	25±3.12c	3	157.51±20.52^{de}	26±3.33^{c}	3	162.38±18.98^{e}	25±3.00^{c}	3	152.78±14.55^{e}
5 Mixed AW	18±2.15^{b}	5	213.33±15.20^{c}	19±2.21^{ab}	5	222.32±13.45^{b}	20±2.98^{b}	5	215.45±14.56^{c}
6 (SD)	15±3.25^{a}	6	238.77±14.67^{b}	15±3.44^{a}	6	206.31±12.34^{c}	17±2.45^{ab}	6	247.56±12.34^{b}
7 (RH)	19±2.38^{b}	4	180.21±14.44^{d}	20±2.18^{b}	4	189.13±16.67^{d}	17±2.67^{ab}	4	179.9±12.22^{d}
8 (Corn)	20±3.10^{bc}	4	112.11±10.46^{e}	21±3.45^{bc}	4	100.09±10.56^{g}	17±3.23^{ab}	4	111.11±12.34^{g}
9 (RS)	22±4.11^{c}	4	100.98±8.9^{f}	23±5.12^{c}	4	89.21±9.99^{h}	20±4.54^{b}	4	109.67±9.89^{g}
10 (Rb)	29±5.15^{d}	2	35.1±8.44^{g}	30±6.71^{d}	2	27.23±8.22^{i}	35±6.23^{d}	2	32.45±4.56^{h}

Notes – Values with the same letter are not significantly different ($p > 0.05$).
1 (SD+ RS)= sawdust + rice straw (1:1)
2 (SD+ RH)= sawdust + rice husk (1:1)
3 (SD + Corn)= sawdust + corn cob (1:1)
4 (SD+ Rb)= sawdust + rubber leaves (1:1)
5 Mixed agricultural wastes) = mixtures of sawdust+ rice straw+ rice husk+ corn cob+ rubber leaves (1:1:1:1:1)
6 (SD)= Sawdust only
7 (RH)= Rice husk only
8 (Corn)= Corn cob only
9 (RS)= Rice straw only
10 (Rb)= Rubber leaves only

Considering other forms of agricultural waste in Thailand as a potential cultivation source represents a key challenge and opportunity, as harmful agricultural waste burning is common in the region. Such burning causes air pollution in Thailand from January to March every year (Bangkok Post 2023). Therefore, there is a lot of potential to reduce such pollution by cultivating mushrooms using agricultural waste instead of burning it. Other opportunities for future research include determining how altering the supplement composition could boost the production yields on sawdust + corn cob substrates.

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References

Avci E, Cagatay G, Avci GA, Suiçmez M et al. 2016 – An Edible Mushroom with Medicinal Significance; *Auricularia polytricha*. *Hittite* Journal of Science and Engineering 3: 111–116.

Bandara AR, Chen J, Karunarathna S, Hyde KD et al. 2015 – *Auricularia thailandica* sp. nov. (Auriculariaceae, Auriculariales) a widely distributed species from Southeastern Asia. Phytotaxa 208: 147–156.

Bandara AR, Karunarathna SC, Phillips AJL, Mortimer PE et al. 2017 – Diversity of *Auricularia* (Auriculariaceae, Auriculariales) in Thailand. Phytotaxa 292:19–34.

Bangkok Post 2023. – "Challenges in tackling PM2.5 crisis." https://bangkokpost.com/opinion/opinion/2548171/challenges-in-tackling-pm2-5-crisis. (Accessed on June 1, 2024).

Cai M, Lin Y, Luo YL, Liang HH et al. 2015 – Extraction, Antimicrobial, and Antioxidant Activities of Crude Polysaccharides from the Wood Ear Medicinal Mushroom *Auricularia auricula-judae* (Higher Basidiomycetes). In International Journal of Medicinal Mushrooms (Vol. 17, Number 6). https://begellhouse.com

Chang ST. 1996 – Mushroom research and development-equality and mutual benefit. Mushroom Biology and Mushroom Products: 1–10.

Chang YS, Lee SS. 2004 – Fungal Diversity Utilisation of macrofungi species in Malaysia. Fungal Diversity 15: 15–22.

Chalermpongse A, Thapyai C, Ramanwong K. 1998 – Biodiversity of Microorganisms at Toe-Daeng Peat Swamp Forest Ecosystems in Narathiwat. Sirinthorn Natural Peat Swamp Forest Research and Study Center Journal 1: 155–166.

Chamratpan S. 2003 – Biodiversity of Medicinal Mushrooms in Northeast Thailand. Proceedings, 2nd International Conference on Medicinal Mushroom and the International Conference on Biodiversity and Bioactive Compounds, Pattaya, Thailand 2003: 271–276.

Choi YJ, Park IS, Kim MH, Kwon B et al. 2018 – The medicinal mushroom Auricularia auricula-judae (Bull.) extract has antioxidant activity and promotes procollagen biosynthesis in HaCaT cells. Natural Product Research 33: 3283–3286.

Choieklin R, Dhitaphichit R. 1999 – iodiversity of macrofungi in the wildlife sanctuary development and reservation extension station, Khao Kheaw, Chonburi. *In*: Baimai, V., Kumhom, R., Chomphuvises, N., Mekruangrussamee, S., Srisawang J., Budhisakollert, U., Rodrungruang, R., Aiemkul, K., Piyottip, S., Treesucon, U., Kitthawee, S. & Milne, J. (Eds.) *Research reports on biodiversity in Thailand*. Biodiversity research and training program, Bangkok, pp. 136–140.

De Silva DD, Rapior S, Fons F, Bahkali AH et al. 2012a – Medicinal mushrooms in supportive cancer therapies: An approach to anti-cancer effects and putative mechanisms of action. Fungal Diversity 55: 1–35.

De Silva DD, Rapior S, Hyde KD, Bahkali AH. 2012b – Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Diversity 56: 1–29.
Duc PH. 2005 – Mushrooms and cultivation of mushrooms in Vietnam. In: Mushroom Growers' Handbook 2 Shiitake Cultivation [Internet]. Seoul, Republic of Korea: MushWorld; p. 260–6. Available from: http://MushWorld.com
Duengkae K. 2006 – Monitoring on species diversity of macrofungi in Khek watershed, Phetchabun Province (in Thai). Songklanakarin Journal of Science and Technology 28: 293–333.
Du P, Cui BK, Dai YC. 2011 – Genetic diversity of wild *Auricularia polytricha* in Yunnan Province of South-western China revealed by sequence-related amplified polymorphism (SRAP) analysis. Journal of Medicinal Plants Research 5: 1374–1381.
Fan L, Zhang S, Yu L, Ma L. 2006 – Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. Food Chemistry, 101(3), 1158–1163.
Hall T. A. 1999 – BioEdit_a_user_friendly_biological_seque. Nucleic Acids Symposium Series 41: 95–98.
Kho YS, Vikineswary S, Abdullah N, Kuppusamy UR et al. 2009 – Antioxidant capacity of fresh and processed fruit bodies and mycelium of *Auricularia auricula-judae* (Fr.) quél. Journal of Medicinal Food 12: 167–174.
Kobayasi Y. 1973 – Enumeration of the Tremellaceous fungi collected in New Guinea. Bulletin of the National Museum of Nature and Science 16: 639–654.
Kobayasi, Y. 1981 – The genus *Auricularia*. Bulletin of the National Science Museum, Tokyo 7: 41–67.
Kornerup A, Wanscher JH. 1978 – Methuen Handbook of Colour. London: Eyre Methuen.
Lowy B. 1952 – The Genus *Auricularia*. *Mycologia* 44: 656–92.
Onyango BO, Palapala VA, Axama PF, Wagai SO et al. 2011 – Suitability of selected supplemented substrates for cultivation of Kenyan native wood ear mushrooms (*Auricularia auricula*). American Journal of Food and Technology 6: 395–403.
Peng JT. 2008 – AGRO-WASTE FOR CULTIVATION OF EDIBLE MUSHROOMS IN TAIWAN [Internet]. Paper presented at the International Workshop on Sustainable Utilization of Biomass and Other Organic Wastes as Renewable Energy Sources and for Agricultural and Industrial Uses. Tagaytay City, Philippines Available from: https://researchgate.net/publication/283117256
Petcharat V, Klingesorn P, Chalemponngse A. 1999 – A field survey and collection of macrofungi In the Ton-Nga-Chang wildlife sanctuary and nearby areas. *In*: Baimai V, Kumhom R, Chomphuvises N, Mekruangrussamee S, Srisawang J, Budhisakollert U, Rodrungruang R, Aiemkul K, Piyottip S, Treesucon U, Kitthawee S. & Milne J. (Eds.) *Research reports on biodiversity in Thailand*. Biodiversity research and training program, Bangkok, pp. 151–154.
Rambaut A. 2012 – FigTree v1.4.0. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/ (Accessed on December 23, 2023)
Sakolrak B. 2006 – Diversity of Mushrooms at Royal Agricultural Station, Angkhang, Chiang Mai Province (in Thai). Master of Science, Kasetsart University, 286 pp.
Sękara A, Kalisz A, Grabowska A, Siwulski M. 2015 – *Auricularia* spp.-mushrooms as novel food and therapeutic agents-a review. Mycology, 67: 1-10.
Silvestro D, Michalak I. 2012 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12: 335-337.
Sun YX, Liu JC, Kennedy JF. 2010 – Purification, composition analysis and antioxidant activity of different polysaccharide conjugates (APPs) from the fruiting bodies of *Auricularia polytricha*. Carbohydrate Polymers 82: 299–304.
Tapingkae T. 2005 – Mushroom growing in Lao PDR. In: Mushroom Growers' Handbook 2 Shiitake Cultivation. Seoul, Republic of Korea: MushWorld; p. 244–59.
Thongklang N, Luangharn T. 2016 – Testing agricultural wastes for the production of *Pleurotus ostreatus*. Mycosphere 7: 766–772.

Thongklang N, Keokanngeun L, Taliam M, Hyde KD. 2020 – Cultivation of a wild strain of *Auricularia cornea* from Thailand. Current Research in Environmental and Applied Mycology 10: 120−130.

Ukai S, Kiho T, Hara C, Morita M et al. 1983 – Polysaccharides in fungi: XIII. Antitumor activity of various polysaccharides isolated from *Dictyophora indusiata*, *Ganoderma japonicum*, *Cordyceps cicadae*, *Auricularia auricula-judae* and *Auricularia* sp. Chemical and Pharmaceutical Bulletin (Tokyo) 31: 741–744.

Verma RK, Verma P. 2017 – Diversity of macro fungi in central India-IV: *Auricularia auricula-judae*, a neutraceutical jelly mushroom. Van Sangyan: Tropical Forest Research Institute [Internet].23–31. Available from: https://researchgate.net/publication/318283696

Walker A, Wannasawang N, Taliam W, Keokanngeun L et al. 2023 – Optimal conditions for mycelial growth and nutritional values of the *Auricularia cornea*. Studies in Fungi 8:19.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990 – Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White, TJ. Eds., PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, 315-322.

Wu F, Tohtirjap A, Fan LF, Zhou LW et al. 2021 – Global diversity and updated phylogeny of *Auricularia* (Auriculariales, basidiomycota). Journal of Fungi 7: 1-71.

Wu F, Yuan Y, Liu HG, Dai YC. 2014a – *Auricularia* (Auriculariales, Basidiomycota): a review of recent research progress. Mycosystema 33: 198–207.

Wu F, Yuan Y, Malysheva VF, Du P et al. 2014b – Species clarification of the most important and cultivated *Auricularia* mushroom "heimuer": Evidence from morphological and molecular data. Phytotaxa 186: 241–53.

Wu F, Yuan Y, Rivoire B, Dai YC. 2015 –Phylogeny and diversity of the *Auricularia mesenterica* (Auriculariales, Basidiomycota) complex. Mycological Progress 14: 1-9.

Yu T, Wu Q, Liang B, Wang J et al. 2023 –The current state and future prospects of Auricularia auricula's polysaccharide processing technology portfolio. Molecules 28: 582.

Yuan Z, He P, Cui J, Takeuchi H. 1998 – Hypoglycemic effect of water-soluble polysaccharide from auricularia auricula-judae quel. on genetically diabetic kk-ay mice. Bioscience, Biotechnology and Biochemistry 62: 1898–1903.

Yuan Y, Wu F, Si J, Zhao YF et al. 2019 –Whole genome sequence of *Auricularia heimuer* (Basidiomycota, Fungi), the third most important cultivated mushroom worldwide. Genomics 111: 50–58.

Zhang X, Bau T, Li Y. 2019 – Biological characteristics and cultivation of *Auricularia fibrillifera*. Mycosystema, 38: 1099-1110.

Zhao S, Rong C, Liu Y, Xu F et al. 2015 – Extraction of a soluble polysaccharide from *Auricularia polytricha* and evaluation of its anti-hypercholesterolemic effect in rats. Carbohydrate Polymers 122: 39–45.