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Article

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First successful domestication of a white strain of *Auricularia cornea* from Thailand

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Abstract

Intraspecies colour variations in cultivated edible mushrooms present novel and potentially valuable alternatives to the research and cultivation industries. In this study, we collected, identified, and domesticated a white strain of *Auricularia cornea* from Thailand. The brown strain of *A. cornea* is one of the top two species of *Auricularia* cultivated and traded in Asia. Since both white and brown phenotypes of *A. cornea* belong to a single species, we established their similarities or differences. Both morphological characteristics and phylogenetic analysis of ITS rDNA sequence data were used to confirm the taxonomic placement of the white *A. cornea* strain in the same clade with the brown *A. cornea*. Nutritional analysis showed that fat, fiber, protein, and total soluble sugar contents of the white *A. cornea* were significantly higher than the commercially used brown strain. The melanin content of the white strain of *A. cornea* (less than 1.5 mg/100g) was not significantly different from that of the brown strain. This discovery may create new opportunities for the mushroom growing industry and for smallholder farmers in Asia.

Key words – colour variation – Melanin – Southeast Asia – wood-ear mushrooms

Introduction

Intraspecies colour variations of cultivated edible mushroom species determine their commercial potentiality. The colour differences among edible mushrooms are also important in identification and edible variability (Williams et al. 2014). For example, the white strain of *Agaricus bisporus* (Lange) Imbach is more commercially popular than the brown strain (Crimini, Portabella) in the USA. For this reason, the white strain accounted for 87% of all domestic *A. bisporus* sales in 2001 (Lucier et al. 2003). Moreover, the white strain and brown strains of *A. bisporus* were shown to possess different nutrient composition (Dikeman et al. 2005, OECD 2007, Phillips et al. 2011).

Among cultivated edible mushrooms, *Auricularia* Bull. is the third most cultivated mushroom in the world (Royse et al. 2017). *Auricularia* is known for its high degree of intraspecies colour

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variation, ranging from brown to red (Mau et al. 1998, Lin et al. 2013). *Auricularia* species have darkly coloured gelatinous basidiomes that are either red (Bandara et al. 2017a) or black (Wu et al. 2014a). Several white strains of *Auricularia* species have been reported from the wild, such as *A. auricula* (L.) Underw. f. *albicans* (Berk.) Kobayasi (Kobayashi 1981), *A. cornea* Ehrenb. (Wong 1989), *A. delicata* (Fr.) Henn. f. *alba* (Kobayashi 1981), *A. fuscosuccinea* (Mont.) Henn. (Sierra et al. 2008), and *A. polytricha* (Mont.) Sacc. f. *leucochroma* (Kobayasi) (Kobayashi 1981). Among these white strains of *Auricularia*, *A. auricula* (Thaithatgoon et al. 2004) and *A. fuscosuccinea* (Mau et al. 1998, Lin et al. 2013) were domesticated for commercial purposes. Nutritional analysis has shown that the cultivated white strain of *A. fuscosuccinea* had a higher proportion of fat, fiber, and protein as compared to the brown strain. Moreover, the white *A. fuscosuccinea* contained lower carbohydrates and reduced sugar levels in comparison with the brown strain (Mau et al. 1998).

Auricularia cornea is an edible mushroom with many medicinal properties (Lowy 1952, Looney et al. 2013). Different ethnic groups have used *A. cornea* as a traditional medicine for anxiety, fear, and poisoning by plant toxins (Fuller et al. 2005, Lampman 2007). *Auricularia cornea* is widespread throughout Asia and was originally described from the Asian Pacific region (Looney et al. 2013). Another study had suggested *A. cornea's* existence in Africa and South America (Wu 2016). Generally, *A. cornea* produces large basidiomes up to 15 cm in width (Lowy 1952, Looney et al. 2013). Our previous study published in 2017 identified that *A. cornea* isolated from Thailand has dark brown abhymenial and hymenial surfaces (Bandara et al. 2017b). It was also reported that the black-brown strain of *A. cornea* has been cultivated in China since 1975 (Zhang et al. 2015).

Commercially cultivated *Auricularia* species in Asia have been primarily identified as *A. polytricha* (Chang & Miles 2004, Yan et al. 2004, Yu et al. 2008, Jia et al. 2011, Razak et al. 2013, Wu et al. 2014a). In 2013, *A. polytricha* was synonymized to *A. nigricans* (Fr.: Fr.) Birkebak, Looney and Sánchez-García which has been found in the New World (Looney et al. 2013). A previous research study from 2000 proposed that the proper name for the cultivated *A. polytricha* should be *A. cornea*, and emphasized the necessity of investigating those species which have been commercially cultivated (Stamets 2000).

Auricularia basidiomes are a rich source of melanin (Zou et al. 2013, Tang & Shi 2014). Approximately 10% of the dry mass of *A. auricula* is known to contain melanin, which is primarily located in the cell wall as dark brown pigments (Prados-Rosales et al. 2015). In comparison, the most abundant mushroom-derived melanin type found in nature, which had been extracted from *A. auricula*, contains eumelanin - a brown to black pigment (Kim et al. 2009, Prados-Rosales et al. 2015, Wu et al. 2018). Melanin isolated from *A. auricula* also possesses anti-microbial or antibiofilm properties, as illustrated by the inhibition of biofilms formed by *Pseudomonas fluorescens* P-3, *Escherichia coli* K-12 and *Pseudomonas aeruginosa* PAO1 (Bin et al. 2012, Bandara et al. 2019). Melanin is found in many different edible mushrooms (Mendoza et al. 1979, De Souza et al. 2018), and is naturally present in some specific sites, such as the dark mucous mass on the mycelium of *Pleurotus cystidiosus* var. *formosensis* (Selvakumar et al. 2008). Moreover, the formation of brown coloured melanin has been observed to cause discolouration of *Agaricus bisporus* basidiomes (Jolivet et al. 1998). Melanin is a product that has applications in the food industry, as well as pharmacological, medicinal, and cosmetic fields (Sun et al. 2016a).

In this study we hypothesized that the white strain of *A. cornea* would have no or a very low concentration of melanin. In addition to testing the melanin content of our strain of *A. cornea*, we provide a full morphological and molecular description of this *Auricularia* species. Furthermore, our study includes a description of the successful domestication process, including substrates and growing conditions.

Materials & Methods

Sample collection

The fresh specimens of white *A. cornea* were collected from a forest in Chiang Mai, Thailand

on August 2016, and macro and micro morphological descriptions were made at the Center of Excellence in Fungal Diversity in Mae Fah Luang University. The specimens were then dried in an electric food dryer at 40-50°C until no more moisture remained, and then sealed in dehydrated silica gel-containing ziplock plastic bags to regulate humidity. The dried specimens in Ziplock bags were then deposited in the Herbarium of Mae Fah Luang University (MFLU).

Morphological characteristics

Description of macroscopic and microscopic morphological characteristics was done by following the methods described in Bandara et al. (2017b). Fresh materials were photographed *in situ* with specific notes on colour notions. Microscopic examinations were done using a Nikon Eclipse 80i (Nikon, Tokyo, Japan) microscope with a camera fitted on the top, and image measurements were taken by Image framework software (Tarosoft®, v0.9.7).

DNA Extraction, PCR, Sequencing, and Phylogenetic analyses

DNA extraction from dried basidiomes was done using Biospin Fungus Genomic DNA extraction kit, BSC14S1 (Bioer Technology Co., Ltd. Bio-Tek, Hangzhou, P.R. China). PCR to amplify internal transcribed spacer (ITS) regions of Nuclear Ribosomal DNA (ITS1-5.8S-ITS2) (White et al. 1990) was carried out as per previously described protocols in Bandara et al. (2017b). The ITS (ITS1-5.8S-ITS2) dataset consisted of 100 isolates. The TABLE 1 contains a list of sequences derived from this study (Accession numbers are in bold) as well as those retrieved from the GenBank, they represent 23 taxa including three outgroup taxa [*Eichleriella deglubens* (FO12006), *Exidia recisa* (FO12006), and *Exidiopsis grisea* (RoKi162)]. Phylogenetic analysis was also done as previously described by Bandara et al. (2017b). The aligned dataset comprised of 573 characters including gaps have been submitted to TreeBASE (submission ID: 24583). Maximum likelihood (ML) analysis was performed by raxmlGUI v1.31 using rapid bootstrap analysis with 1000 replicates in the GTR+I+G model (Silvestro & Michalak 2012). In the ML analysis, bootstrap support >80 was considered strong, between 60-80 was considered moderate, and less than 60 was considered poor.

Table 1 Information of the sequences used in the phylogenetic analyses. Collection numbers of the newly produced sequences and species names of type species are in **bold**. The country of collection is referred to in parentheses using the first English letter of the country's name following the **bold** collection number.

Taxon name	Herbarium code	Collection number	GenBank accession ITS	References
Auricularia angiospermarum	BJFC017274	Cui12360	KT152097	Wu et al. (2015a)
A. asiatica	BBH895		KX621160	Bandara et al. (2017b)
A. brasiliana	URM85567	AN-MA 42	KP729275	Wu et al. (2015b)
A. Americana		PBM2295	DQ200918	Looney et al. (2013)
A. auricula-judae		JT14	KT152101	Wu et al. (2015a)
A. cornea	TENN066990	PBM3754	JX065164	Looney et al. (2013)
A. cornea	PDD103780		KR336701	Bandara et al. (2015)
A. cornea	PDD97684		KR336700	Bandara et al. (2015)
A. cornea	PDD94825		KX621146	Bandara et al. (2017b)
A. cornea	PDD92640		KR336699	Bandara et al. (2015)
A. cornea	MFLU162109	ST10	KX621143	Bandara et al. (2017b)
A. cornea	MFLU130403	AB30	KX621145	Bandara et al. (2017b)
A. cornea	MFLU162108	AB113	KX621140	Bandara et al. (2017b)
A. cornea	MFLU162110	ST14	KX621141	Bandara et al. (2017b)
A. cornea	MFLU162104	MRC8	KX621144	Bandara et al. (2017b)
A. cornea	MFLU162107	AB51	KX621142	Bandara et al. (2017b)
A. cornea	LE262938		KJ698424	Malysheva & Bulakh (2014)
A. cornea	LE269791		KJ698435	Malysheva & Bulakh (2014)

Table 1 Continued.

Taxon name	Herbarium code	Collection number	GenBank accession ITS	References
A. cornea		MFUAB36	KR336702	Bandara et al. (2015)
A. cornea		AG1547	KX022016	Wu (2016)
A. cornea		Dai12587	KX022012	Wu (2016)
A. cornea		Dai13547	KX022013	Wu (2016)
A. cornea		Dai15336	KX022014	Wu (2016)
A. cornea		AG6	KX022015	Wu (2016)
A. cornea	MFLU 190691	**AB58** (T)	MK610683	This study
A. cornea	MFLU 190692	**AB110** (T)	MK610684	This study
A. cornea	MFLU 190693	**AB111** (T)	MK610685	This study
A. cornea	MFLU 190694	**AB114** (T)	MK610686	This study
A. cornea	MFLU 190695	**DSK5** (T)	MK610687	This study
A. cornea	MFLU 190696	**GH09** (I)	MK610687	This study
A. cornea	MFLU 190697	**KUN43** (C)	MK610689	This study
A. cornea	MFLU 190698	**MY01** (M)	MK610690	This study
A. cornea	MFLU 190699	**MY02** (M)	MK610691	This study
A. cornea	MFLU 190700	**MY03** (M)	MK610692	This study
A. cornea	MFLU 190701	**MY04** (M)	MK610693	This study
A. cornea	MFLU130419	**PA109** (P)	MK610694	This study
A. cornea	MFLU 190702	**ST03** (T)	MK610695	This study
A. cornea	MFLU 190703	**ST02** (T)	MK610696	This study
A. cornea	MFLU 190704	**ST01** (T)	MK610697	This study
A. cornea (white strain)	MFLU 190705	**V-11** (T)	MK610698	This study
A. cornea	MFLU 190706	**VI03** (V)	MK610699	This study
A. cornea	MFLU 190707	**VI05** (V)	MK610700	This study
A. delicata Clade-I	MFLU162112	MRC7	KX621149	Bandara et al. (2017b)
A. delicata Clade-I	MFLU162119	AB104	KX621147	Bandara et al. (2017b)
A. delicata Clade-II	TENN016963	AJS1304	JX065158	Looney et al. (2013)
A. fuscosuccinea	TENN058951	TFB10743	JX065141	Looney et al. (2013)
A. heimuer	BJFC	Dai13765	KM396793	Wu et al. (2014b)
A. heimuer		Dai2291	KM396785	Wu et al. (2014b)
A. heimuer		Dai11936	KM396787	Wu et al. (2014b)
A. heimuer		2008-2	KM396783	Wu et al. (2014b)
A. heimuer		Heishan A	KM396782	Wu et al. (2014b)
A. heimuer		Dai11935	KM396786	Wu et al. (2014b)
A. heimuer		Cui6137	KM396784	Wu et al. (2014b)
A. heimuer		Heishan	KM396778	Wu et al. (2014b)
A. heimuer		Heishan 2	KM396779	Wu et al. (2014b)
A. heimuer		Heishan 3	KM396780	Wu et al. (2014b)
A. heimuer		Xiaoheimao	KM396781	Wu et al. (2014b)
A. heimuer	MFLU 190708	**KUN02** (C)	MK610701	This study
A. heimuer	MFLU 190709	**KUN31** (C)	MK610702	This study
A. heimuer	MFLU 190710	**KUN32** (C)	MK610703	This study
A. heimuer	MFLU 190711	**KUN34** (C)	MK610704	This study
A. heimuer	MFLU 190712	**KUN35** (C)	MK610705	This study
A. heimuer	MFLU 190713	**KUN41** (C)	MK610706	This study
A. heimuer	MFLU 190714	**KUN52** (C)	MK610707	This study
A. heimuer	MFLU 190715	**KUN53** (C)	MK610708	This study
A. heimuer	MFLU 190716	**SK02** (S)	MK610709	This study
A. mesenterica	BRNM706955		KP729278	Wu et al. (2015b)
A. minutissima	BJFC017994	Dai14881	KT152104	Wu et al. (2015a)
A. nigricans	TENN056825	TFB4405	JX065172	Looney et al. (2013)
A. orientalis	BJFC	Dai14875	KP729270	Wu et al. (2015b)
A. polytricha		ZH	HM448450	Jia et al. (2011)
A. polytricha		781	HM448451	Jia et al. (2011)

Table 1 Continued.

Taxon name	Herbarium code	Collection number	GenBank accession ITS	References
A. polytricha		Shanghai3	HM448452	Jia et al. (2011)
A. polytricha		951	HM448453	Jia et al. (2011)
A. polytricha		243	HM448454	Jia et al. (2011)
A. polytricha		Qingyou	HM448455	Jia et al. (2011)
A. polytricha		Sanyou	HM448456	Jia et al. (2011)
A. polytricha		Zimuer	HM448457	Jia et al. (2011)
A. polytricha		Hupo	HM448458	Jia et al. (2011)
A. polytricha		Au2	HM448459	Jia et al. (2011)
A. polytricha		Sumao3	HM448460	Jia et al. (2011)
A. polytricha		Chuan er7	HM448461	Jia et al. (2011)
A. polytricha		Hongdamuer	HM448462	Jia et al. (2011)
A. polytricha		Ap1142	HM448463	Jia et al. (2011)
A. polytricha		Ap1192	HM448464	Jia et al. (2011)
A. polytricha		3039	HM448465	Jia et al. (2011)
A. polytricha		Ap117	HM448466	Jia et al. (2011)
A. polytricha		Apw82	HM448467	Jia et al. (2011)
A. polytricha		Kang1	HM448468	Jia et al. (2011)
A. polytricha		Juer2	HM448469	Jia et al. (2011)
A. polytricha		Shanghai1	HM448470	Jia et al. (2011)
A. polytricha		Huang er10	HM448471	Jia et al. (2011)
A. scissa	TENN059729	TFB11193	JX065160	Looney et al. (2013)
A. subglabra	TENN058607	TFB10499	JX065155	Looney et al. (2013)
A. thailandica	MFLU130410		KR336693	Bandara et al. (2015)
A. tibetica	BJFC017181	Cui12267	KT152106	Wu et al. (2015a)
A. villosula	LE 296422		KJ698418	Malysheva & Bulakh (2014)
Eichleriella deglubens		FO12006	AF291272	Weiß & Oberwinkler (2001)
Exidia recisa		MW315	AF291276	Weiß & Oberwinkler (2001)
Exidiopsis grisea		RoKi162	AF291281	Weiß & Oberwinkler (2001)

(C): China, (I): Indonesia, (M): Myanmar, (P): Philippines, (S): South Korea, (T): Thailand, (V): Vietnam

Isolation, Spawn production, and Basidiome production

Pure mycelium cultures of the white strain of *A. cornea* were established from MFLU 190705 (V-11) using tissue culture on potato dextrose agar (PDA) (Oxoid, CM0139). The mycelia were subcultured at 28°C for 14 days and transferred to cooked wheat grain and incubated at 28°C for three weeks. Basidiomes were produced in plastic bags with Oak (*Quercus* sp.) sawdust (particle size ≤ 3 mm) mixed with rice bran in a 78:20 ratio. Calcium oxide (1%) and Calcium sulphate (1%) were added to the mixture and mixed with water until the moisture level reached about 65%. The substrate mixtures were packed (1 kg per bag) tightly in plastic bags and autoclaved at 121°C and 15 psi for 4 h. The bags were allowed to cool for 24 h and then inoculated with 1% (w/w) of mycelia colonised rye grains. Inoculated bags were incubated at 28°C in the dark. Once the substrate was fully invaded by mycelia, the bags were completely opened. The temperature for the fruiting phase was 28°C and the humidity was 85–95%. Mushroom yield was recorded for each flush for 30 bags, which were randomly selected from 200 bags. The biological efficiency of each mushroom bag was calculated using the following equation (Liang et al. 2019).

Biological efficiency (%) = Yield of fresh basidiome (g)/Substrate dry weight (g) × 100

Brown strain MFLU 190697 (KUN43) of *A. cornea* was used as a control for the experiment.

Quantification of nutrient content

Proximate analysis of the fresh samples of cultivated brown and white strains of *A. cornea* was carried out as per the standard procedure of the Association of Official Analytical Chemists (AOAC 1995). As outlined in (Bandara et al. 2017a), the moisture, crude ash, crude fat, crude fiber

and crude protein contents were characterized. Total Soluble Sugars were analyzed by using the phenol-sulfuric acid method (Nielsen 2010).

Extraction, Precipitation, and Characterization of melanin

Extraction – Melanin was extracted from the dried, cultivated white strain of *A. cornea* samples containing 10-12% moisture content by following the method used in Zou et al. (2010) with slight modifications. The basidiomes of *A. cornea* were pulverized and sieved through a 40-mesh sieve. Approximately 6 g of powdered basidiome were washed with running water at a ratio of 30 ml/g (water/raw materials) for 5 minutes (min), followed by centrifugation at 4000 rpm for 5 min. The supernatant was discarded and the precipitates were immersed in water. Then, the water/solid ratio was adjusted to 43 ml/g and the pH was adjusted to 12.0 with 1 M NaOH. The suspension was placed in a brown bottle, which was placed in an ultrasonic instrument for ultrasound extraction at a power of 240 W and a temperature of 63°C for 40 min. After that, each sample was centrifuged at 4000 rpm for 5 min and the supernatant containing melanin was collected and stored at 4°C in the dark.

Precipitation – The supernatant was adjusted to pH 2.0 with 3M HCl to precipitate melanin, followed by centrifugation at 4000 rpm for 30 min at the room temperature. The supernatant was discarded and the precipitated melanin was washed successively with chloroform, ethyl acetate, and ethanol. Finally, the solid melanin was dissolved in 0.01 M NaOH. The solution was stored in a brown bottle.

Characterization: An ultraviolet (UV) spectrophotometer (Shimadzu UV-2401PC) was used to record UV spectra of the melanin extracted from the samples and the synthetic melanin (Sigma Chemicals Co. St. Louis, USA).

Quantification of melanin content

Approximately 4 mg of synthetic melanin (Shanghai Macklin Biochemical Co. Ltd, China) was weighed and dissolved in 100 ml 0.01M NaOH solution. Then, the stepwise dilution method was used to prepare five concentrations (20 μg/mL, 16 μg/ml, 4 μg/ml, 1.6 μg/ml and 0.8 μg/ml). Using 0.01M NaOH solution as a blank control, the UV absorbance value was determined at 400 nm. A standard curve was drawn (concentration vs absorbance) in Microsoft Excel to achieve the following formula: $y = 0.0116x + 0.0401$; $R^2 = 0.9983$. The melanin extracted from six mushroom samples was dissolved in 25, 30, 20, 25, 35, and 25 ml 0.01M NaOH. Different sample solutions were determined using the UV spectrophotometer at 400 nm following the steps outlined above. Finally, the formula obtained using the standard curve was used to determine the content of melanin in different samples.

Statistical analysis

Thirty mushroom bags were used to determine basidiome production, and three replicates of brown and white strains of *A. cornea* samples from first three flushes were used for nutrient and melanin quantification analyses. The results were analyzed using R statistical software version 3.5.1 (RCoreTeam 2014) Welch's two sample t-tests were performed to observe whether there was a significant difference between the nutrient content of the dark brown and white *A. cornea* at $p < 0.05$.

Results

Morphological characteristics

The morphological characteristics of the white strain of *A. cornea* are shown in Fig. 1. The basidiome and internal features of white strain of *A. cornea* identified in this study are as follows:

Basidiome – 2–5 cm, short stalks, orbicular to cupulate to auriculiform; in fresh state abhymenial, hymenial surfaces white (4A1); in dry state abhymenial surface yellowish white (4A2); hymenial surface light yellow (4A4), ridges and veins present on abhymenial surface.

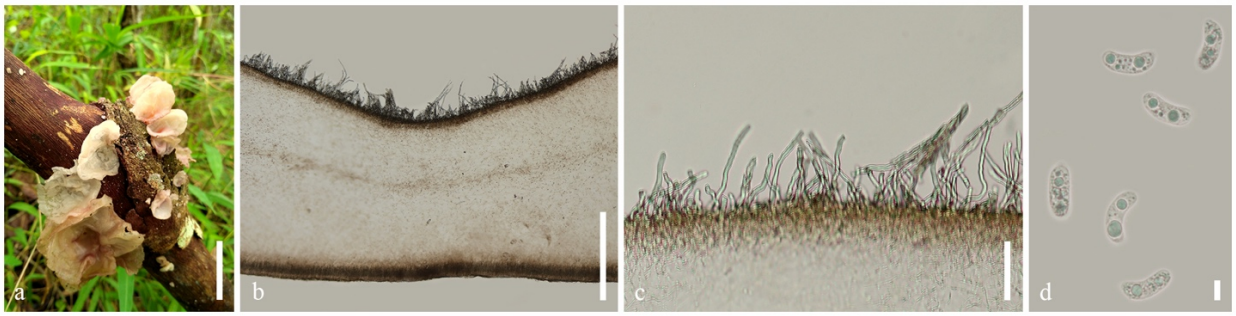

Fig. 1 – Photographs of white strain of *Auricularia cornea* (MFLU 190705). a *Auricularia cornea* growing on a decaying wood in the wild. b Cross-section of the basidiome. c Abhymenial hairs. d Basidiospores. Scale bars: a = 3 cm, b = 500 μm, c = 100 μm, d = 5 μm.

Internal features – thickness 920–1220 μm; medulla present; abhymenial hairs densely arranged, fasciculated at the bottom, separated or clustered at the top, hyaline, tip blunt; clamp connections present; zona pilosa 100–230 μm; zona compacta 20–30 μm; zona subcompacta superioris 30–35 μm; zona laxa superioris 295–330 μm; medulla 173–178 μm; zona laxa inferioris 315–750 μm; zona subcompacta inferioris 50–75 μm; hymenium 80–90 μm; basidia 55–66 × 3–4 μm, cylindrical, blunt or tapered ends, sterigmata occasionally observed; basidiospores (12.3)13.6–15.5(17) × (4.5)4.9–5.6(6) μm, $\bar{x}$ ± SD = 14.5 ± 0.97 × 5.2 ± 0.36 μm, Q = 2.4–3.3 (n = 30), allantoid, smooth, hyaline, with one large irregularly shaped guttule.

Phylogeny

43 ITS sequences which were identical to other sequences in the alignment were excluded from the maximum likelihood analysis. Strains corresponding to the excluded sequences were shown with equal (=) sign in Fig. 2. After identical sequences had been eliminated, 57 sequences were retained in the resulting ITS alignment. Nineteen terminal groups were obtained that corresponded to phylogenetic species in the analysis of ITS data (Fig. 2). Eighteen species were recovered as monophyletic. *Auricularia delicata* was found to be paraphyletic or polyphyletic. All cultivated strains of *Auricularia* from different countries were grouped into *A. cornea* or *A. heimuer* with moderate to strong bootstrap supports (Fig. 2). The ITS sequences of the white strain of *Auricularia* (V-11) were found to be exactly identical to Kun43, which is *A. cornea* (Fig. 2).

Isolation, Spawn production, and Basidiome production

The mycelium of the white strain of *A. cornea* was grown on sawdust mixed supplements medium (1kg of medium per bag, 65% moisture content) and was fully colonized within 52.9 ± 1.2 days. The average yield of the first three flushes on the sawdust mixed supplements medium was 57.6 ± 5.6 g. The biological efficiency was 16.5 ± 1.2% (Fig. 3).

Nutrient content

The proximate composition, total soluble sugar content, and melanin content of the brown and white strains of *A. cornea* are summarized in Table 2.

Characteristics and quantity of melanin found in *Auricularia cornea*

The UV-visible absorption spectra (190–400 nm) of melanin solutions (pH 12) isolated from *A. cornea* in contrast to the control synthetic melanin sample are shown in Fig. 4. The melanin extracted from basidiomes of *A. cornea* brown strain, white strain, and synthetic melanin each showed similar optical absorbance in a wide spectral range, as shown in Fig. 4. The quantification of melanin extracts from *A. cornea* samples indicated that they contained <1.5 mg/100g of melanin content. The melanin content of brown and white strain *A. cornea* was recorded as 0.46 ± 0.37 and 1.49 ± 0.46 mg/100 g of dry weight, respectively. However, we did not observe any significant differences in melanin quantity between the brown and white strains.

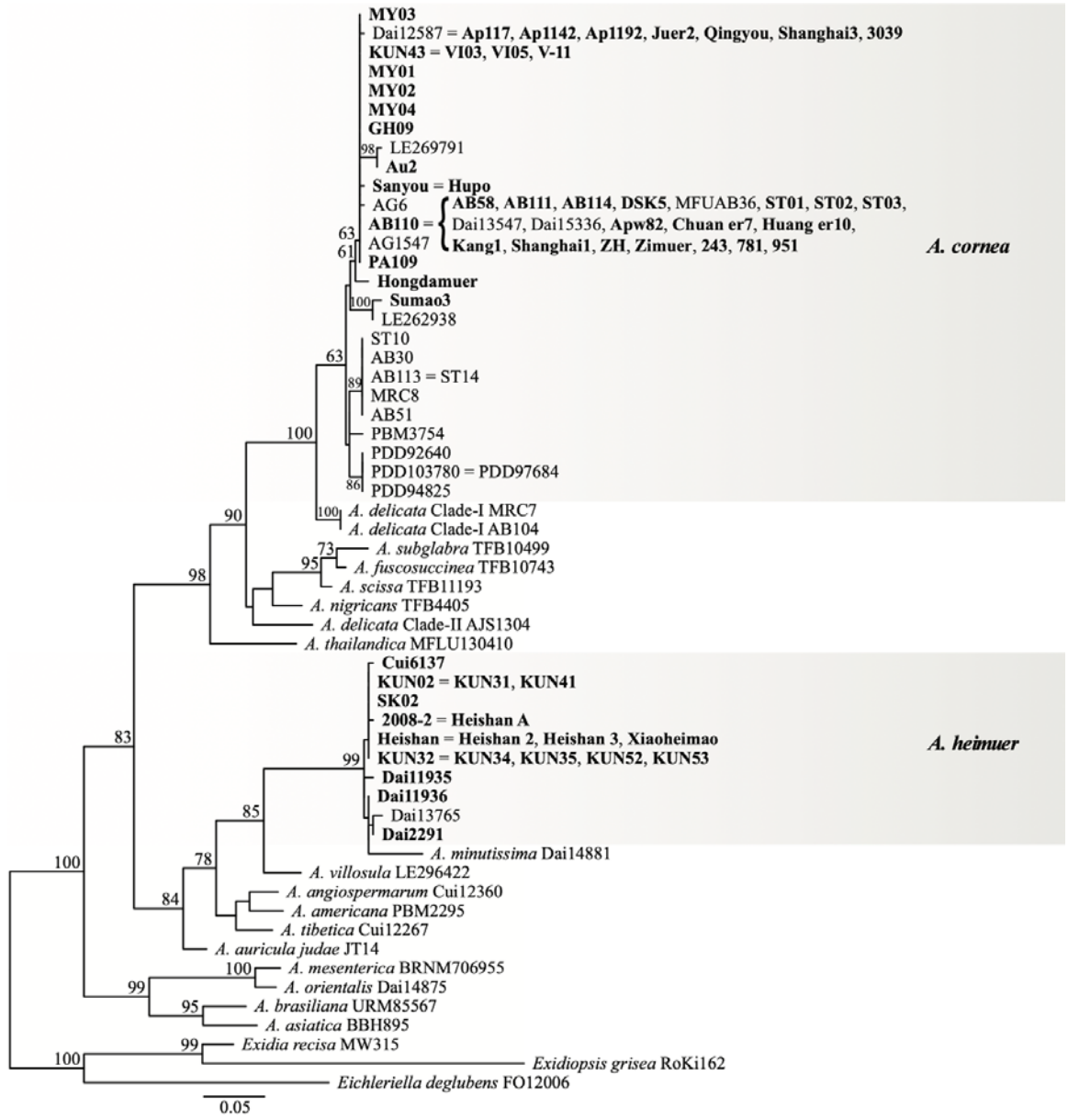

Fig. 2 – Phylograms inferred from maximum likelihood analyses of ITS sequences of *Auricularia*. Herbarium codes or collection codes are followed by species names. Cultivated strains are in bold. Bootstrap support values >60% are displayed above each node. The tree is rooted with *Eichleriella deglubens* (FO12006), *Exidia recisa* (MW315), and *Exidiopsis grisea* (RoKi162).

Fig. 3 – Structural differences of two developmental stages of cultivated white *Auricularia cornea*. a Cultivated basidiomes of white *Auricularia cornea* on sawdust medium. b The primordia stage. c The mature stage of basidiomes. Scale bars: b = 1 cm, c = 2 cm. The biological efficiency was calculated for these samples.

Table 2 Proximate composition, total soluble sugar content (% of dry weight) and melanin content (mg/100g) of basidiome of *Auricularia cornea* brown and white strains. Superscripts ([a, b]) refer to significant differences between mean values determined at $p < 0.05$ using Welch's two sample t-tests.

Nutrient constituent	Brown strain	White strain
Moisture	80.75±0.20[w]	82.25±0.32[w]
Crude ash	1.89±0.06 [a]	3.75±0.11 [b]
Crude fat	0.99±0.39 [a]	2.37±0.30 [b]
Crude protein	6.57±0.18 [a]	13.85±0.04 [b]
Total dietary fiber	87.47±0.26 [a]	72.57±0.23 [b]
Total soluble sugar	6.94±0.54 [a]	9.03±0.30 [b]
Melanin	0.46±0.37	1.49±0.46

[w] Represented as % of fresh weight, protein conversion factor (N × 6.25)

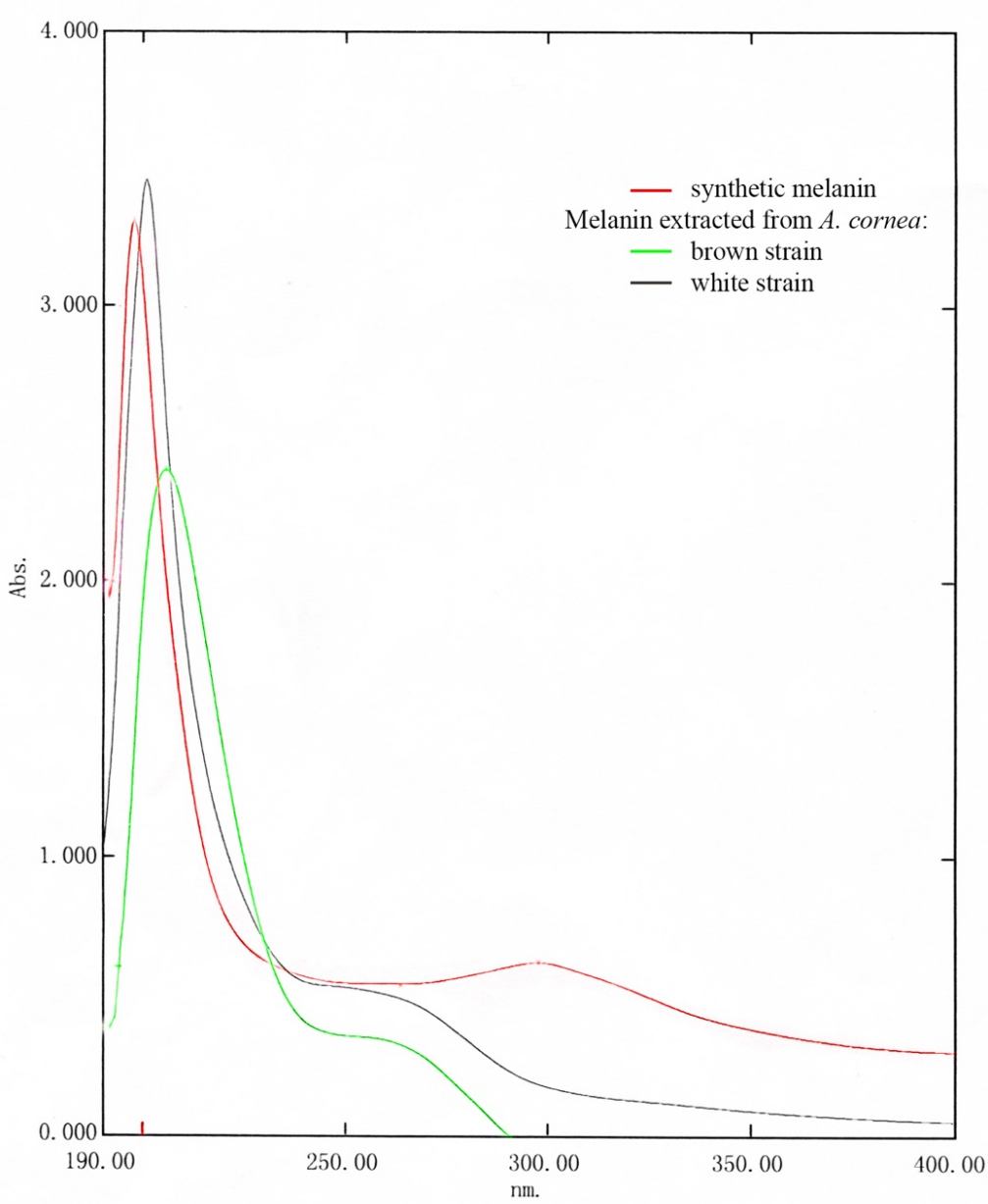

Fig. 4 – Comparison of ultraviolet-visible (UV) absorption spectra of synthetic melanin and melanin extracted from basidiomes of *Auricularia cornea*. The graph illustrates the UV absorption spectrums of synthetic melanin (red) and melanin extracted from basidiomes of *Auricularia cornea* brown strain (green) and white strain (blue).

Discussion

In this study, a white strain of *A. cornea* collected from a forest in Thailand was successfully identified and domesticated. This is the first report of a white strain of *A. cornea* being domesticated from a tropical region, which has the potential to be cultivated in warm climatic conditions. Consistent yield of the white strain of *A. cornea* was achieved between bags under 200 bags of production. The biological efficiency of the white strain of *A. cornea* (16.5 ± 1.2%) was lower than that of the brown strains of *A. cornea*, which previously have been commercially cultivated (Tan et al. 2018). This difference in biological efficiency was likely due to the fact that the brown strains of *A. cornea* were cultivated on a substrate with optimum C/N ratio, while the other required supplements which have been characterized previously (Tan et al. 2018). However, the white mutant of *A. cornea* gave a 12.4% higher yield compared with its purple black coloured wild-type grown in China (Wang et al. 2015). As we now have domesticated the white strain of *A. cornea*, this study provides the opportunity to further optimize its cultivation conditions and increase its biological efficiency for higher industrial quality.

The complete ITS region, including the ITS1, 5.8S and ITS2 of rDNA, was used in this study for the phylogenetic analysis of the white strain of *A. cornea*. ITS has been established as an adequate molecular marker for phylogenetic species delimitation in *Auricularia* that correspond with their morphology (Weiß & Oberwinkler 2001, Looney et al. 2013, Malysheva & Bulakh 2014, Wu et al. 2014b, Bandara et al. 2015). According to morphological characteristics (e.g. hyphal zonation, length of the abhymenial hairs) and rDNA ITS analysis of *Auricularia* samples collected from different countries in Asia, including the white strain, *A. cornea* is commercially cultivated in China, Indonesia, Myanmar, Philippines, South Korea, Thailand, Vietnam. However, in most studies, the cultivated varieties of *A. cornea* have been misidentified as *A. polytricha* (Yan et al. 2004, Yu et al. 2008, Du et al. 2011a, 2011b, Jia et al. 2011, Razak et al. 2013), which has been synonymized as *A. nigricans* (Looney et al. 2013).

Nutritional analysis conducted in this study shows that the white strain of *A. cornea* contains a significantly higher ($p < 0.05$) proportion of fat, protein, and soluble sugars than the brown strain by dry weight, suggesting the potentially high demand for this strain. However, our analysis shows that the total dietary fiber content in the white strain of *A. cornea* is lower than that of the brown strain, which may limit its usability as a fiber-rich edible mushroom when compared to the brown strain. Previous studies have also demonstrated examples of white and brown strains of *Auricularia* sp. having different proximate contents. For example, significant higher contents of protein and fiber have been reported from the white strain of *A. fuscosuccinea* than its brown strain (Mau et al. 1998). Moreover, the fat quantities were similar on both brown and white strains of *A. fuscosuccinea* (Mau et al. 1998).

Mushrooms are highly valued as a good source of protein, which is an essential part of the human diet (Ingram 2002). The newly domesticated white strain of *A. cornea* mushroom contains higher amounts of protein than currently commercially cultivated *Auricularia* species (Crisan & Sands 1978, Mau et al. 1998, Cao et al. 2017, USDA 2018a, 2018b). Lipid content of mushrooms is limited (2–6%), due to a low total lipid content and a low proportion of desirable n-3 fatty acids (Kalac 2009). Compared to the commercially cultivated *Auricularia* species (Crisan & Sands 1978, Mau et al. 1998, Cao et al. 2017, USDA 2018a, 2018b), the white strain of *A. cornea* domesticated in our study contained lower fat content. Therefore, the current study also contributes to the knowledge of the proximate content diversity of cultivated *Auricularia* in South Asia.

Edible mushrooms are rich in non-starch polysaccharides, which can be a good source of dietary fiber for humans (Cheung 2013). Most non-starch polysaccharides values were reported in the literature based on crude fiber content (Crisan & Sands 1978, Aletor 1995, Mau et al. 1998, Bandara et al. 2017a). However, few studies have reported *Auricularia* species as having higher dietary fiber content than other edible mushrooms (Cheung 1997, 2013, Sekara et al. 2015). In this study, more than 70% of the dry weight of total dietary fiber content was reported from both white and brown strains of *A. cornea*. Therefore, *A. cornea* can be considered as an ideal source of dietary fiber, which can support health maintenance and disease prevention. Although a significantly

higher quantity of total soluble sugar content was found in the *A. cornea* white strain than its brown strain, both strains are low in soluble sugars in contrast to the other edible *Auricularia* species (Mau et al. 1998, Kadnikova et al. 2015, Bandara et al. 2017a). The lower quantity of soluble sugars may result in mild taste differences in all edible *Auricularia*, including *A. cornea*. The genetic similarity coefficient was 0.636 between the white mutant and wild-type and 0.788 between the light brown mutant and its wild-type, which indicates a large genetic difference between the mutant and its wild-type (Wang et al. 2015)

The optical absorbance spectra for melanin extracted from *A. cornea* strains showed profiles quite similar to that of synthetic melanin, suggesting that the quality of the melanin extracted from the *A. cornea* strains were as high as the synthetic melanin. Previous studies have also shown similar UV-visible absorbance spectra from melanin extracts of *A. auricula-judae* (=*A. auricula*) (Zou et al. 2010, Sun et al. 2016b, Hou et al. 2019). The melanin quantity extracted appears to be dependent on the species. For instance, 120.05 mg/100 g of melanin could be yielded under optimized conditions from *A. auricula-judae* (Zou et al. 2010). Also, melanin quantity of *A. auricula-judae* affected by the substitute materials used for cultivation (Yao et al. 2019). As this is the first study which characterized the quantity of melanin in *A. cornea*, it is unknown whether the lower melanin content observed in the study was due to actually lower melanin content compared to other *Auricularia* sp. or due to the substitute materials used for cultivation. Nevertheless, this study provides the ground for further optimization of the melanin extraction conditions for future studies.

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