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Agaricus subrufescens: new to Thailand

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ABSTRACT

Agaricus subrufescens is an edible and medicinal mushroom of *Agaricaceae, Agaricales* originally described from North America. It has increasingly been used as a health food and alternative medicine and is also used in cosmetics. *Agaricus subrufescens* has been shown to exhibit many pharmaceutical traits such as anti-cancer, anti-microbial and immunomodulatory properties and is rich in bioactive compounds, especially β-glucans. Recently, four specimens of *Agaricus subrufescens* were collected from northern Thailand and are the first records of this taxon for Thailand and surrounding regions. The data from macro- and microscopic features are consistent with the characteristics of the species. The molecular data indicates the Thai collections cluster in *A. subrufescens sensu lato*. The species is reported here because of the significance of finding this very important taxon in Thailand which has both commercial and biotechnological potential.

Keywords: *Agaricaceae,* medicinal fungus, new record

1. INTRODUCTION

Agaricus subrufescens (*Agaricaceae*, *Agaricales*) was discovered in north eastern America [1-2] and has also been found in Hawaii, Israel and Taiwan [3]. The species typically grows in clusters or occasionally singly on soil following rain. *A. subrufescens* has unique characters that are useful in its identification, such as odor, spore size, spore shape, and the form of its cheilocystedia and pileipellis. Kerrigan [3] provided details of the history, nomenclature and typification of this species.

A. subrufescens was first published by C.H. Peck [1]. It has also been found in south America, Brazil in 1960. The Brazilian

mushroom is commonly referred as *A. blazei* and *A. brasiliensis* rather than *A. subrufescens* and controversy has existed over the correct name of *A. subrufescens*. Wasser [4] rejected conspecificity between *A. brasiliensis* and *A. subrufescens* due to the features of spore and cheilocystedia. However, new data indicated that *A. brasiliensis* and *A. subrufescens* are biologically and phylogenetically the same species [3].

There have been few reports on the diversity of the genus *Agaricus* in Thailand. We have been collecting and studying the genus *Agaricus* in northern Thailand and have numerous collections which are presently under study. The first report from these studies was on a closely related genus by Zhao et al [5] who reported on *Micropsalliota* in northern Thailand based on morphological and molecular data. We have also found many *Agaricus* species which we are presently studying and have also collected *A. subrufescens*. In this paper we report on collections of this important cultivatable and medicinal species that we have made in Thailand, including micro- and macro- characters. Our identification is also confirmed using molecular data. This is the first report of *Agaricus subrufescens* in Thailand.

Mushrooms of the genus *Agaricus* have been commercially cultivated for a long time. *Agaricus bisporus* is one of the most widely cultivated mushrooms worldwide, and occurs in section *Agaricus* [6]. Wild edible mushrooms in section *Arvenses* include *A. albolutescens, A. arvensis, A. silvicola, A. augustus and A. subrufescens*. *A. arvensis* is the "horse mushroom" which is collected and eaten in Europe. *A. silvicola* is also consumed and is popular in Europe. Nevertheless, heavy metal accumulation in all species has been reported [7]. Cadmium, lead and mercury were built up in the fruiting bodies of these species. Wild *A. augustus* is collected for consumption in Canada, some parts of Mexico and the USA. *A. albolutescens* is also edible but there are few scientific articles on this taxon. *A. subrufescens* (commonly called *A. brasiliensis*) is also commercially cultivated in Brazil, Japan and Taiwan [3] and has previously been reported to have pharmaceutical potential [2] and is used in cosmetics [8]. Bioactive compounds extracted from *A. subrufescens* are regarded as a good candidate as anti-cancer [9, 10] and biomodulatoratory agents [11]. *A. subrufescens* bioactive compounds have been isolated from their fruiting bodies [9, 10], or culture extraction from pure culture of mycelia [12]. β-glucan is a constituent of the cell wall of fungi, also including *A. subrufescens*. It is a well-known immunomodulating substance which indirectly suppresses tumor cells [13].

Although this is a well known edible species we questioned northern Thai locals in neighborhood markets concerning the edibility of this species. The questionnaires indicated that *A. subrufescens* is not available in the local markets and is not generally eaten. This is an important medicinal mushroom that could be introduced to Thailand for cultivation and medicinal use. The aim of the present study is to report and illustrate the new findings of this medicinal species in Thailand. Due to its possible value in biotechnology, it is important to and obtain local isolates that can be used in breeding improvement experiments for cultivation.

2. MATERIALS AND METHODS

2.1 Sampling

Agaricus fruiting bodies were collected in nature from Chiang Mai and Chiang Rai provinces during rainy seasons of 2004 to 2010 [14]. We made four collections from four different sites under forest trees. The dried studied materials are deposited in the Mae Fah Luang University herbarium,

BIOTEC Bangkok Herbarium (BBH) and San Francisco State University (SFSU).

2.2 Macroscopic and Microscopic Characterization

Photographs and other data such as odor were recorded in the field in order to document macroscopic characters. The morphology of the pileus, lamellae and stipe were recorded in the laboratory. The microscopic identification of all specimens was carried out under a compound microscope. All macro-morphological characters were described based on fresh material and documented by photographs. Color designations (e.g. 4B5) are from Kornerup and Wancher [15], while the color name with the first letters capitalized (e.g. grayish yellow) are from Ridgeway [16]. Specimens were dried and placed in heat-sealed plastic bags separately. For micro-morphological examination, sections were cut with a razor blade from dried specimens and mounted on slides in 5% KOH and Congo red, and then observed, measured and illustrated under a compound microscope (Olympus CX41). In the description of the basidiospores: n indicates the number of spores which were measured. Lm is the mean spore length over a population of spores. Wm is the mean spore width over a population of spore. Q is the length/width ratio (L/W) of a spore in side view.

2.3 DNA Extraction, PCR and Sequencing

Dried mushroom samples were used to extract genomic DNA using the CTAB method (hexadecryltrimethylammonium) [17]. DNA concentrations were estimated visually in 1% agarose gel containing ethidium bromide by comparing molecular weight and band intensity with a DNA ladder 1,000 BP (Transgen Biotech). The PCR reactions were performed in a 40 ml volume (0.20 mM primers; ITS5: 5'-GGAAGTAAAAGTCG TAACAAG G-3' and ITS4: 5'TCCTCCGC TTATTGATATGC3') [18]; 10-20 ng DNA template, 1x buffer, 4 mM dNTPs, 0.25 units Taq and sterile water). The thermal cycles consisted of 95°C for 5 min, followed by 30-35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min and 30 seconds, with a final extension step of 72°C for 5 min. The PCR products were verified by staining with ethidium bromide on 1% agarose electrophoresis gels stained with ethidium bromide in 1x tris-boric acid EDTA buffer. ITS5 and ITS4 were used to sequence both strands of the DNA molecules at INRA, Bordeaux, France, The University of Hong Kong and National Botanic Gardens Belgium.

2.4 Sequence Alignment and Phylogenetic Analysis

The taxon information and GenBank accession numbers used in the molecular analysis are listed in Table 1. Sequences for each strain were aligned using Clustal X [19]. Alignments were manually adjusted to allow maximum sequence similarity. Gaps were treated as missing data. Maximum Likelihood (ML) and unweighted maximium Parsimony (MP) analysis were performed using PAUP* 4.0b10 [20]. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa [21]. Trees were viewed in Treeview and exported to graphics programs [22].

Table 1. List of sequenced specimens with GenBank accession number used in the phylogenetic analysis

No.	Identification	Country of origin	Genbank	Other codes	Reference
01	*A.* subrufescens	Hawaii	AY818646		Kerrigan 2005
02	*A. subrufescens*	Hawaii	AY818647		Kerrigan 2005
03	*A. subrufescens*	Hawaii	AY818648		Kerrigan 2005
04	*A. subrufescens*	Brazil	AY818653		Kerrigan 2005
05	*A.* subrufescens	Japan	AY818660		Kerrigan 2005
06	*A.* subrufescens	New York	EU071699		Kerrigan 2007
07	*A.* subrufescens	Brazil	AY818651		Kerrigan 2005
08	*A.* subrufescens	Brazil	AY818652		Kerrigan 2005
09	*A.* subrufescens	China	AY818655		Kerrigan 2005
10	*A.* subrufescens	California	AY818656		Kerrigan 2005
11	*A.* subrufescens	California	AY818657		Kerrigan 2005
12	*A.* subrufescens	Brazil	AY818658		Kerrigan 2005
13	*A.* subrufescens	Sylvan proprietary hybrid	AY818659		Kerrigan 2005
14	*A.* subrufescens	Thailand		BBH19514	Zhao et al. 2011
15	*A.* subrufescens	Thailand		BBH19522	Zhao et al. 2011
16	*A.* subrufescens	Thailand		MFLU10-0065	Zhao et al. 2011
17	*A.* subrufescens	Thailand		MFLU10-0662	Zhao et al. 2011
18	*A.* subrufescens	Hawaii		DEH513	Kerrigan 2005
19	*A.* rufotegulis	UK	AY818649		Kerrigan 2005
20	*A.* brasiliensis	Brazil	AY818650		Kerrigan 2005
21	*A.* augustus	California	AY484672		Geml 2004
22	*A.* augustus	California	AY484671		Geml 2004
23	*A.* fissuratus	Denmark	AY484683		Geml 2004
24	*A. bisporus*	Hungary	AY484694		Geml 2004

2.5 ITS1 + 2 DNA Sequence Analysis

The dataset comprises of 5 *A. subrufescens* sequences where 4 sequences belong to Thai *A. subrufescens* and one is Hawaiian extracted from GenBank. The samples studied had an rDNA ITS1 & 2 sequence with nominal length of 698 nt; however, due to frequent length polymorphisms it could vary. Alignment was done using the ClustalW algorithm, followed by inspection and manual correction. All sequences comprise data from both strands, except two Thai sequences BBH19514 and BBH19522.

3. Results

Four collections of *A. subrufescens* were made from northern Thailand during extensive surveys in forests between 2004-2010 (Figure 1). The collections are described and illustrated, and differences compared with other closely related collections. Two sequences of nuclear rDNA of the four Thai specimens were compared with a sequence of Hawaii *A. subrufescens*, DEH513, deposited in GenBank, due to the geographical similarity. The samples studied had an rDNA ITS1 & 2 sequence with nominal length of about 698,

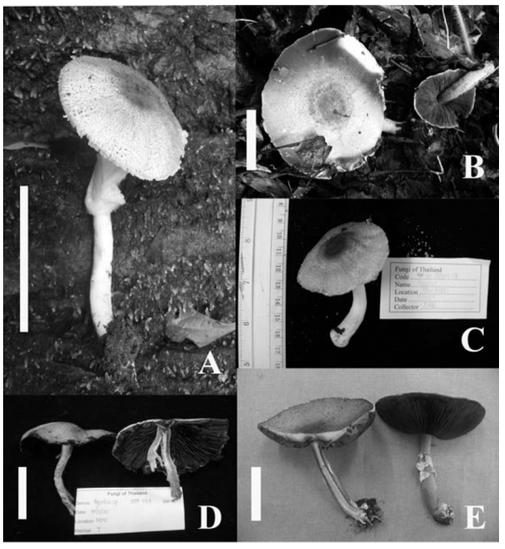

Figure 1. Basidiocarps of all *A. subrufescens* collections from northern Thailand. A: BBH19522, B-C: MFLU10-0065, D: MFLU10-0662 and E: BBH19514. Scale bars A-E = 5 cm.

but this could vary due to frequent length polymorphisms. All collections cluster with *A. subrufescens* retrieved from GenBank and nest in section *Arvenses*.

Agaricus subrufescens Peck, Annual Report New York State Museum 46: 25 (Figure 1A-E).

3.1 Macroscopic and Microscopic Identification

Basidiocarp medium to large (Figure 1). Pileus 50-93 mm diam., circular,hemispherical, convex to plano-convex with a flattened disc, edge straight/ appendiculate/ denticulate with remnants of veil, surface dry, covered by fibrillose squamulose hairs, appressed and dense at the centre, less dense towards the margin, purple-brown (6E5)/ reddish-brown (8E4-8E5)/ dark brown (9F6) at the center and purple-brown (6E5)/ reddish-brown (8E4-8E5)/ brownish orange (7C2) towards the margin. Context 4-6 thick at the disc, white, fleshy, cottony. Lamellae attachment free, crowded, with 3-4 series of lamellae, 3-7 mm broad, normal, white/ pink/ brown/ dark brown with age. Stipe 68-95 × 6-12 (base 12-15) mm, upwardly tapering or cylindrical, subbulbous with rhizomorphs, insertion central, pith hollow, surface smooth, withnscattered small fibrillose nodules below annulus. Annulus membranous, pendent, superior, white and lost in age. Smell slightly almond. The KOH reaction is negative or yellow.

Basidiospores (Figure 2b) 4-6.5 × 3-4.5 μm, ellipsoid, some ovate, smooth, apiculus distinct, without germ pore. Basidia (Figure 2c) 10-19 × 4-8 μm, clavate, tetrasporic, hyaline, smooth. Cheilocystidia (Figure 2d) consisting of 7-18 × 5-13 μm elements, catenulate, hyaline, smooth, spherical or subspherical, 2-4 elements in chains, pyriform in basal element. Pileipellis (Figure 2e) a cutis composed of 3.75-16.25 μm hyphae, repent in net-like, brown or light brown, smooth, constricted at the septa in most cases. Annulus consisting of 4-7.5 μm diam. hyphae, mostly cylindrical, smooth and hyaline.

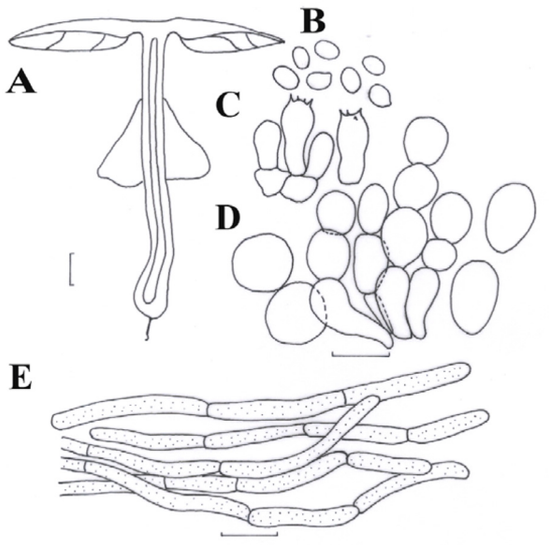

Figure 2a-e. Line drawing of BBH19422; a: basidiome, b: basidiospore, c: basidia, d: cheilocystedia and e: pileipellis, scale bar = 10 μm.

Habitat:

solitary or clustered in soil with *Bambuseae* sp. and *Dipterocarpus* sp. and *Samanea saman* (Jacq.) Merr. and in flower pot.

Distribution:

Hawaii, Israel, North America, Taiwan, Thailand (this study).

Material examined:

THAILAND, Chiang Mai Province, Mae Taeng District, Ban Pha Deng Village, Mushroom Research Centre, N 19°17.123' E 98°44. 009', elev. 900 m., in flower pot, 28 August 2004, K.D. Hyde, ZRL2134 (BBH19514, SFSU); *ibid.* Mok Fah Waterfall, N 19°08' 43.20" E 98°54' 19.52", elev. 1464m, near bamboo, 25 June 2005, R.L. Zhao, ZRL2036 (BBH19422, SFSU); Chiang Rai Province, Tambon Mae Korn and Tambon Huay Chompoo, Muang District, Khunkorn Waterfall, N19°51-54' E 99°35.39', elevation 1208 m, moist upper mixed deciduous forest (Royal Forest Department, 1962), 15 August 2009, S.C. Karunarathna (MFLU10-0065); *ibid*, Tambon Thasud, Muang District, Mae Fah Luang University, N 18° 05' 59.1", E 102° 40' 02.9", elevation 488 m, under *Samanea saman* (Jacq.) Merr, 17 August 2010, P. Sysouphanthong (MFLU10-0662).

The micro and macro-morphological characters of collections of *A. subrufescens* in northern Thailand were similar (Table 2) and comparable with the holotype of the species [1, 3]. Peck [1] reported only macro-morphological characters in his article. Later, Kerrigan [3] reported both of macromorphological and micro-characters for Peck's specimen. Kerrigan [3] also suggested that morphology is variable and the variation e.g. in cap shape, vigor and cuticle pigment, is dependent on the genotype and environmental factors. We also found variability in cuticle pileus pigmentation, ranging from purple-brown, reddish-brown, dark brown to brownish orange. Although the morphology of this species is somewhat variable, Kerrigan [3]

Table 2. Synopsis of collections of *Agaricus subrufescens* from northern Thailand compared with the holotype.

Specimen code	Habitat	Collector and year	Basidiocarp	Odor	Chielocystedia	Basidiopore	Pileipellis
BBH19514	Solitary in rich soil of flower pot	Hyde K.D., 2004	Plano-convex, purple-brown (6E5),fibrillose squamulose	Slightly almond-liked	8-18× 7-12 µm, catenulate, ellipsoid, spherical, sub spherical, hyaline, smooth	5-6.5×4-4.5µm, ellipsoid with undistinct appendage,brown	A cutis composed of hyphae of 10-15 µm in diam, hyaline or yellowish brown
BBH19422	Solitary in soil near the bamboo	Zhao R., 2005	Plano-convex, reddish brown (8E4) fibrillose squamulose	Slightly almond-liked	7-13 × 7-11 µm, catenulate, sphere, sub spherical, hyaline, smooth	4-5×3-3.5µm, ellipsoid, some ovate, apiculus distinct	A cutis composed of hyphae5-7.5µm in diam, brown or light brown
MFLU10-0065	Three fruiting bodies grouped in soil under *Dipterocarpus* sp.	Karunarathna S.C., 2009	Convex/plano-convex, reddish brown (8E5) fibrillose squamulose	Slightly almond-liked	8-12× 7-13 µm, spherical, sub spherical, hyaline, smooth	4.5-6×3-4µm, ellipsoid, smooth,hyaline	A cutis composed of hyphae6-8.5µm in diam, brown
MFLU10-0662	Solitary in soil under *Samanea saman* (Jacq.) Merr.	Sysouphanthong P., 2010	Hemispherical, brownish orange (7C2)/ dark brown (9F6) fibrillose squamulose	Unknown	8-18 × 5-11 µm, catenulate, spherical, sub-spherical, hyaline, smooth 7.5-13.5 µm broad,	4-5×3-4µm, ellipsoid, smooth, apiculus distinct, hyaline	A cutis composed of hyphae 3.75-16.25µm in diam, brown or light brown
Holotype	Pile of leaves	Peck C.H. 1893	Deeply hemispherical, becoming convex or plano-convex, whitish, grayish or dull reddish, silky fibrillose, squamulose	Almond-liked	Catenulate, cylindrical, clavate, swollen with a few to many spherical	6.1-7.1×4.1-5.1 µm, ellipsoid, smooth, hyaline	10-26µm broad, inflated elongate and occasional subglobose element

described the variation range of some characters, e.g. cap shape, cheilocystidia shape, number of sterigma, and spore size. Our specimens fit well within the range of characters reported (Table 2). One of the most unique characters is the almond-like odor, present in our specimens, with the exception of MFLU10-0662, indicating that they are *A. subrufescens*. However, to confirm our morphological identification we also compared ITS sequence of Thai taxa with ITS sequence dataset of *Agaricus* section *Arvenses*.

The ITS sequence dataset comprised of 24 fungal strains (Table 1) that comprise 20 sequences from GenBank and four sequences from tropical areas . All the selected taxa of *A.garicus* belong to the section *Arvenses*, while *A.garicus bisporus* which belongs to the sect. *A.garicus* was chosen as the out group. Out of the 583 characters, 492 characters were constant, 55 were variable but parsimony-uninformative, and 36 were parsimony-informative. The MP tree was produced after 1002003 rearrangements and the score of best tree found was 103. This MP tree was chosen to represent the phylogenetic position of the new species (Figure 3). In the phylogenetic tree, the new record *A. subrufescens* from Thailand forms a monophyletic group with the *A. subrufescens* from Hawaii with the support of 93% bootstrap showing the tropical similarity of the *A. subrufescens*.

Discussion

Macroscopic, microscopic as well as molecular approach confirms that the collections from Thailand belong to *A. subrufescens* in the section *Arvenses*. The section

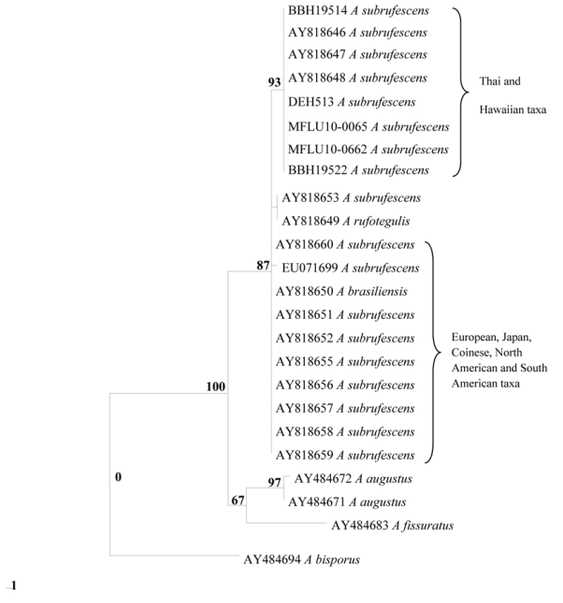

Figure 3: Maximum parsimony phylogram showing the phylogenetic position of Thai *Agaricus subrufescens* with some selected *Agaricus* species from the sect. *Arvenses* based on ITS rDNA sequences. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Values above the branches are parsimony bootstrap (e" 50%). The tree is rooted with *A. bisporus* belongs to the sect. *Agaricus*.

Arvenses contains 19 defined species within six sub-groups: *Aestivalis*, *Arvensis*, *Augustus*, *Macrosporus*, *Spissicaulis* and *Salvicola* [5, 23].

Phylogenetic analyses of ITS-rDNA sequence data shows that all Thai collections cluster with *A. subrufescens* retrieved from GenBank and nest in section *Arvenses* indicate that Thai taxa belong to *A. subrufescens*. The Thai specimens are closely related to Hawaiian taxa (DEH513, AY818646, AY818647 and AY818648) forming an monophyletic group with *A. subrufescens* from Hawaii with the bootstrap support of 93% showing the geographic similarity of *A. subrufescens* and the data suggests that Thai and Hawaiian samples belong to a very distinct and isolated population or to an intersterile entity. The Hawaiian members were different from European, Japanese, Chinese, North American and South American taxa showing a separate clade with 87% support of bootstrap. All the *A.subrufescens* from tropical and temperate areas have grouped with *A.augustus* and *A fissuratus* giving 100% bootstrap support. ITS sequence based phylogenetic tree shows that the tropical taxa clustering in one population, significantly varied from other isolates.

In accordance with the geographical similarities and resemblance of ITS sequences, we compared all Thai strains with one Hawaii ITS sequence, DEH 513. Table 3 shows all variable position of nucleotides among all 5 ITS1 & 2 regions of the nuclear rDNA sequences. The ITS sequences from the Thai specimens have slight differences among themselves. One Thai specimen (BBH19514) shows identical characters to DEH513, while the other three Thai collections were most similar to Hawaiian specimen, but have slightly different positions of nucleotides i.e. polymorphic positions. There are two

Table 3: Variable position within ITS1+2 sequences of *A. subrufescens*

Specimens	Origin	ITS1+2 polymorphic positions					
		96	122	125	264	529	579
BBH19514	Thailand	T	T	T	T	T	T
BBH19522	Thailand	T	T	T	T	Y	Y
MFLU10-0065	Thailand	T	C	C	C	T	T
MFLU10-0662	Thailand	W	Y	Y	T	T	T
DEH513	Hawaii	T	T	T	T	T	T

polymorphic positions at 529 and 579 in the sequence of BBH19522. Within the sequence of MFLU10-0065, there are three prominent polymorphic positions at the nucleotides 122, 125, 264. MFLU10-0662 shows polymorphism positions at the nucleotides 96, 122 and 125. Kerrigan [3] has indicated that the three ITS sequences from Hawaiian specimens had different characteristics at the positions 281 and 478 and were one nucleotide shorter than the other at the position 485 among the non-tropical *A. subrufescens*. The Hawaiian sequences were distinguished uniquely from Europe, North America and South America taxa by polymorphism positions [3]. All Thai specimens have the same characters with the Hawaii sequence at these polymorphism positions. Thus, the Thai specimens may be different from the strains from North America, which are basal. This may indicate that the Thai strains are not

identical to temperate strains. However, further studies with many more sequences and strains are needed to establish relationships of strains within the species. The data however, suggests that Thai and Hawaiian samples belong to a very isolated population or to an intersterile entity. The exact status of this group should be carefully examined following many more collections.

Conclusions

The data obtained from the present studies confirmed that our Thai collections are *A. subrufescens*. This is the first discovery of the species in Thailand. Macro- and micro-characters characters are consistent with the characteristics of this *A.garicus* species. We are also established relationships of Thai collections with a Hawaiian taxa by comparing the ITS sequencesFurther studies of more collections of this species are needed to establish the variability of this taxon.

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References

[1] Peck C.H., New York State. *Mushroom Ann. Rep.*, 1893; **46**: 83-152.

[2] Firenzuoli F., Gori L., Lombardo G., The medicinal mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems, *eCAM.*, 2008; **5(1)**: 3-15.

[3] Kerrigan R.W., *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms, *Mycologia*, 2005; **97(1)**: 12-24.

[4] Wasser, S.P., Didukh, M.Y., de Amazonas, M.A.L., Nevo, E., Stamet, P. and da Eira, A.F., Is a widely cultivated culinary-medicinal royal sun *Agaricus* (the Himematsutake mushroom) indeed *Agaricus blazei* Murrill?, *Int. J. Med. Mushrooms*, 2002; **4**: 267-290.

[5] Zhao R.L., Desjardin D.E., Soytong K., Perry B.A., Hyde H.D., A monograph of *Micropsalliota* in northern Thailand based on morphological and molecular data, *Fungal Diver.*, 2010; **45(1)**: 33-79.

[6] Challen M.P., Kerrigan R.W., Callac P., A phylogenetic reconstruction emendation of *Agaricus* section *Duploannulatae*, *Mycologia*, 2003; **95(1)**: 61-73.

[7] Kalač P., Svoboda L., A review of trace element concentrations in edible mushrooms, *Food Chem.*, 2000; **69**: 273-281.

[8] Hyde K.D., Bahkali A.H., Moslem M.A., Fungi an unusual source for cosmetic, *Fungal Diver.*, 2010; **43**: 1-9.

[9] Itoh H., Ito H., Hibasami, H., Blazein of a new steroid isolated from *Agaricus blazei* murrill (himematsutake) induces cell dealth and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells, *Oncol. Rep.*, 2008; 20: 1359-1361.

[10] Kim C.F., Jiang J.J., Leung K.N., Fung K.P., Lau C.B.S., Inhibitory

effect of *Agaricus blazei* extracts on human myelomia cells, *J. Ethnopharmacol.*, 2009; **122**: 320-326.

[11] Chan Y., Chang T., Chan C.H., Yeh Y.C., Chen C.W., Shieh B., Li C., Immunomodulatory effects of *Agaricus blazei* murrill in Balb/cByJ mice, *J. Microbiol. Immunol. Infect.*, 2007; **40**: 201-208.

[12] Kasai H., He L.M., Kawamura M., Yang P.T., Deng X.W., Munkanta M., Yamashita A., Terunuma H., Hirama M., Horiuchi I., Natori T., Koga T., Amano Y., Yamaguchi N., Ito M., IL-12 production induced by *Agaricus blazei* fraction H (ABH) involves Toll-like Receptor (TLR), *eCAM.*, 2004; 1(3): 259-267.

[13] Ohno N., Furukawa M., Miura N.N., Adachi Y., Motoi M., Yadomae T., Anti-tumor β-glucan from the cultured fruit body of *Agaricus blazei*, *Biol. Pharm. Bull.*, 2001; **24(7)**: 820-828.

[14] Kumla J., Danell E., Bussaban B., Lumyong S., Suitable growth conditions and nutrition factors on *In vitro* culture of *Phlebopus portentosus* (Boletales), *Chiang Mai J. Sci.*, 2011; **38(1)**: 156-159.

[15] Kornerup A., Wancher J.H., *Methuen Handbook of Color*, London: Methuen, 1978.

[16] Ridgeway R., *Color Standard and Color Nomenclature*, Baltimore, 1912.

[17] Zolan M., Pukkila P., Inheritance of DNA methylation in *Coprinus cinereus*, *Mol. Cell. Biol.*, 1986; **6(1)**: 195.

[18] White T.J., Bruns T., Lee S., Taylor J., Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, In: Innis M.A., Getland D.H., Sninsky J.J., White T.J., (Eds), *PCR Protocols: A Guide to Methods and Applications. Academic Press*, San Diego, 1990: 315-322.

[19] Thompson J.D., Gibson T.J., Plewnick F., Jeanmougin F., Higgin D.G., The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acid Res.*, 1997; **25**: 4876-4882.

[20] Swofford D.L., *PAUP*: Phylogenetic Analysis Using Parsimony (and other methods)*, Sunderland, MA: Sinauer Associates, 1998.

[21] Felsenstein J., Phylogenies and the comparative method, *Am. Nat.*, 1985; **125(1)**: 1-15.

[22] Page R.D., Tree view: An application to display phylogenetic trees on personal computers, *Comp. Appl. Biosci.*, 1996; **12**: 357-358.

[23] Calvo-Bado L., Noble R., Challen M.P., Dobrovin-Pennington A., Elliot T.J., Sexuality and genetic identity in the *Agaricus* section *Arvenses*, *Appl. Environ. Microbiol.*, 2000; **66:** 728-734.

[24] Kerrigan R.W., Callac P., Guinberteau J., Challen M.P., Parra L.A., *Agaricus* section *Xanthodermatei*: a phylogenetic reconstruction with commentary on taxa, *Mycologia*, 2005; **97(6)**: 1292-1315.