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Morphological characteristics and molecular identification of a wild Thai isolate of the tropical mushroom Hed Taen Rad (*Macrocybe crassa*)

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Abstract. *Inyod I, Sassanarakit S, Payapanon A, Keawsompong S. 2017. Morphological characteristics and molecular identification of a wild Thai isolate of the tropical mushroom Hed Taen Rad (*Macrocybe crassa*). Biodiversitas 18: 221-228.* Hed Taen Rad or *Macrocybe crassa* is a wild edible mushroom found in Thailand. It has a large fruiting body with a meaty texture and a delicious taste. Five strains of Hed Taen Rad; DOA, DOA-1, DOA-4, DOA-7 and DOA-10 were tested in different media, pH values and temperatures to measure mycelium growth kinetics and identify mycelia and basidiomata. Results indicated that mushroom mycelial growth rates were highest on MEA medium at pH values between 8 and 10, and temperatures between 20°C and 30°C. Identification of the isolates was based on the morphology characteristics of the basidiomata were closely similar to *M. crassa* (Berk.), and different from *M. gigantea* (Massee). For molecular analysis by sequencing of their internal transcribed spacer (ITS) regions was identified as a *T. giganteum* since limited ITS rDNA sequences from *M. crassa* exist in public databases. Therefore, the identification of five strains of Hed Taen Rad using molecular methods could be erroneous. Therefore, morphology characteristics study is suitable for further analysis method on genetic study of Hed Taen Rad.

Keywords: Hed Taen Rad, *Macrocybe crassa*, molecular analysis, morphological characteristics

INTRODUCTION

Wild mushrooms are becoming more important in our diet for their nutritional, organoleptic and pharmacological characteristics (Solak et al. 2006; Diez and Alvarez 2001). Hed Taen Rad (*Macrocybe crassa* or *Tricholoma crassum*) can be found throughout Thailand and in neighboring countries. It has different names depending on location such as Hed Tub Tao Khaow (Central region), Hed Jun (Northern), and Hed Taen Had or Hed Yai (Northeastern) (Petcharat 1996). Hed Taen Rad is usually found on ground with leaf accumulation, and in forests or mountainous areas. More fruiting bodies are found during the rainy season, with average relative humidity of 70% and temperature range 28-30°C. Natural *M. crassa* is generally rather expensive and rare, because it is usually found only once a year, particularly in the rainy season (Teaumroong et al. 2002).

Payapanon and Srijumpa (2008) reported that 10 isolates of Hed Taen Rad were collected between 2005 and 2006, and five isolates were obtained in 2007 from their natural habitats in the Northern, Northeastern, Central, Eastern and Southern areas of Thailand. They were classified into three morphological groups based on the diameter of the pileus and their stipe length ratio. Chingdaung et al. (1986) reported that most strains from various sources including Chiang Mai and Kanchanaburi Provinces had a medium-sized cap and caespitose basidiomata. Compared to strains collected from Chonburi and Tak Provinces, the fruiting bodies occurred singly, and strains from Tak Province had the smallest pileus. Petch (1912) noted that a revised account of *T. crassum* (Berk.) Sacc., was based on material collected in the Kandy District of Central Province, Sri Lanka. He placed another species that was also described from Sri Lanka, *T. pachymeres* (Berk. & Broome) Sacc., in synonymy. This caused confusion between the two species that has persisted to this day. Therefore, an advanced method needs to be developed for the identification of many mushroom species.

Several recent studies have shown that molecular identification can be successfully used for fungi (Arnold et al. 2007; Ligrone et al. 2007; Morakotkarn et al. 2007). In the biotechnological field, molecular genetic markers have been applied for rapid identification of different kinds of mushrooms (Froslev et al. 2007). The combination of morphological studies and molecular phylogenetic analysis is, therefore, a good tool to identify mushroom species. Accurate taxonomic identification and phylogenetic classification of mushrooms would be helpful in various mushroom species and could assist information transfer for genetic engineering or commercial cultivation of important species in the future (Mello et al. 2006).

The objectives of this study combined morphological classification and analysis of phylogenetic relationships within species, based on the sequences of their internal transcribed spacer (ITS) region to resolve the taxonomy of *M. crassa* in Thailand.

MATERIALS AND METHODS

Mushroom isolates

Five significantly high production yield isolates: DOA, DOA-1, DOA-4, DOA-7 and DOA-10 were obtained from the Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. All isolates were collected from their natural habitats in the Northern, Northeastern, Central, Eastern and Southern areas of Thailand. Pure cultures were transferred into PDA slants and maintained at room temperature.

Morphological analysis

Morphological study of mycelia

Mycelial discs of each isolate were collected from the edges of actively growing colonies, placed onto a suitable medium in 90 mm Petri dishes and incubated at room temperature (28-30°C). Colony morphology was observed after 7 days incubation at room temperature and the characteristics of the fungi and other biological features were recorded, measured and photographed under a compound microscope. At least 50 characteristics were examined for each isolate.

Growth of mushroom mycelia on different media and pH levels

Factorial experiments were conducted in completely randomized design (CRD) with four replications. Factor A used media types of potato dextrose agar (PDA), corn meal agar (CMA), coconut water agar (CWA), malt extract agar (MEA), V-8 juice agar and glucose peptone agar. Factor B used media pH levels as follows: pH 4, 5, 6, 7, 8, 9, and 10. All media were prepared separately and adjusted to pH levels with either HCl or NaOH. All five isolates of *M. crassa* were cultured on PDA for 7 days, and then agar plugs were cut from actively growing colony margins with a cork borer and transferred to the middle plate in each tested media and pH level. The inoculated plates were measured to obtain data on colony diameters (cm) and mycelial density. The data were analyzed for variance by Duncan's multiple range test (DMRT) at $P = 0.05$.

Effect of temperature levels on mycelium growth

Experiments were conducted using CRD with four replications. All five isolates were grown on suitable media at room temperature for 5 days. Five-millimeter diameter agar plugs were removed with a sterile cork borer from the leading edge of colonies, and one plug was placed on plates containing 20 ml of medium. MEA was prepared and adjusted to pH 4, 5, 6, 7, 8, 9, and 10 and also evaluated at temperatures of 15, 20, 25, 30, 35, and 40°C. The inoculated plates were checked to obtain data on colony diameters (cm) and mycelial density. The data were analyzed for variance by DMRT at $P = 0.05$.

Morphological characterization of basidiomata

The macroscopic characteristics of the basidiomata collected from each isolate were recorded, following the technique of Largent (1977). For microscopic analysis, the dry materials were rehydrated in 70% ethanol, followed by either 5% KOH or Melzer's reagent (Largent et al. 1977). The basidiocarps were rehydrated by soaking in water for 10 min before analyzing their morphology. Qualitative characteristics such as the color and shape of the pileus, color of stipes, and color of mushroom spore print were evaluated by eye. For microscopic characteristics, free-hand transverse sections 0.1 mm thick were made from rehydrated basidiocarps using a sharp surgical blade. The sections were immersed in a diluted solution of methyl blue stain and left for 10 min. The thinnest sections were selected, placed on glass slides and covered with cover slips. Low-power (×40) objectives of a standard light microscope were used to observe the basidia in the sections; colors and sizes of the basidiospores were also determined.

Molecular identification of Hed Taen Rad

DNA extraction

Basidiospores were suspended in sterile distilled water and counted with a hemocytometer. Spore concentrations were adjusted to 10^5 spores per ml. About 20,000 spores were inoculated onto PDA medium and incubated at 25°C. Basidiospore germinations were observed with a compound microscope, and the colony of each isolate was transferred onto new PDA medium and used for DNA analysis. The mycelium was harvested by filtration through filter paper. DNAs were extracted following the protocols of Lee and Taylor (1990) with some modifications. Extraction buffer (1% CTAB, 0.7 M NaCl, 50 mM Tris-HCl, 10 mM EDTA, pH 8) was added to the fungal samples. The mycelium was frozen with liquid nitrogen and ground to a fine powder. The volume was then adjusted by adding 700 µL of extraction buffer, mixed by inverting the tubes and incubated at 65°C for 1 hour. Samples were centrifuged at 12,000×*g* for 10 min at 25°C. The upper liquid phase was transferred to a new microcentrifuge tube containing 7.5 M ammonium acetate and DNA was precipitated by ethanol. The pellet was redissolved in 50 µL of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA pH 8.0).

Polymerase Chain Reaction (PCR) amplification

Polymerase Chain Reaction (PCR) amplification products for sequencing the ITS region were obtained using two pairs of universal primers ITS5 and ITS4 (White et al. 1990). Amplification was performed in a 50 µL reaction mix: 10 mM of each dNTP (1 µL), 10 µM of each primer (1 µL), 10% of dilution buffer (5 µL), 25 mM of Mg (5 µL), 4 M of enhancer (5 µL) and 60-62% of sterile distilled water (30.8 µL). Then, 0.2 µL of the *Taq* DNA polymerase kit from FERMENTAS and 10-50 ng of genomic DNA template (1 µL) was carried out using a PCR Model MJ Research DYAD ALD in a 200 µL reaction tube. Amplified products were checked by electrophoresis in 1% agarose gels with 0.003% ethidium bromide in 0.5×TBE buffer.

DNA purification and sequencing

PCR products were purified using a NucleoSpin® Extract Kit (Macherey-Nagel, Germany). The PCR products were sequenced by Macrogen. Inc. in Korea with the same primers used in the PCR amplification.

Phylogenetic relationship analysis

To determine the phylogenetic relationships, sequence analysis of the ITS regions of the rDNA repeats were performed and data compared to related species retrieved from GenBank. For each fungal isolate, sequences generated from the ITS/5.8 gene together with a reference sequence obtained from GenBank were aligned using CLUSTALX (1.83) (Thompson et al. 1997). Bioedit (Hall 1999) was used to obtain an assembled sequence. Alignment was manually adjusted to allow maximum alignment and minimize gaps. Phylogenetic analyses were based on maximum parsimony (MP) performed for the ITS in PAUP*4.0b10 (Swofford 2002). Clade stability was assessed by a bootstrap analysis with 1000 replicates, each with ten replicates of random stepwise addition of taxa.

RESULTS AND DISCUSSION

Morphological analysis

Morphological study of mycelia

Mycelium growth of all five isolates was circular in shape on PDA. The colonies had white irregular edges, were flattened and thick, and grew over the full PDA plates within 10-18 days. Under a compound microscope, the hyphae were hyaline and septate with many clamp connections. Hypha width varied from 2-6 µm.

Different media including PDA, CMA, CWA, MEA, V-8 juice agar and glucose peptone agar with different pH levels were used for culturing all five strains of Hed Taen Rad. Results revealed that the rate of mycelial development was affected by both the culture medium and pH, with significant differences between the acidic and alkaline treatments. The best mycelial growth was found on MEA medium at pH 8, 9, and 10 with very abundant mycelial density (Figure 1). Results showed that the mycelium of almost all strains of Hed Taen Rad presented a growth of 8.50 cm (fully colonized by mushroom mycelium in Petri dishes) for 12 days. Nasim et al. (2001) reported that the mycelial growth of *Pleurotus ostreatus*, varieties *sajor-caju*, *citydeosus* and *Volvariella volvacea* were maximized in medium plates containing MEA, but the mycelial growth rate was slow on glucose peptone agar at all pH levels compared to other media. Romero-Arenas et al. (2012) reported that the strain *P. ostreatus* CP-50 presented varying degrees of tolerance to alkaline pH in PDA and yeast complete medium (YCM) with different growth rates, and the development and morphology of their colonies in alkaline pH were not significantly affected. The ability of the fungus to utilize nutrients for mycelial growth may be affected in different media and at different pH values, with the possibility that they may be toxic at the concentrations used (Carlile et al. 2001), and the pH of the culture was an important parameter that affected fungal morphology and mycelial density (Gibbs et al. 2000). Goldberg and Williams (1991) reported that the most favorable pH for mushroom growth was between 7 and 8, similar to results obtained here. Incubation temperatures of 35°C and 40°C caused total mycelium growth inhibition of all tested strains, and these temperatures did not favor fungus growth. All five strains grew well at 20-30°C, similar to results reported by Quimio et al. (1990) who recommended 22°C to 30°C for *Agaricus bisporus* growth. Fungal development may be accelerated or inhibited by temperature (Miles and Chang 1997). Thus, it was possible to verify a temperature range for Hed Taen Rad growth in spawn production and possibly in compost cultivation.

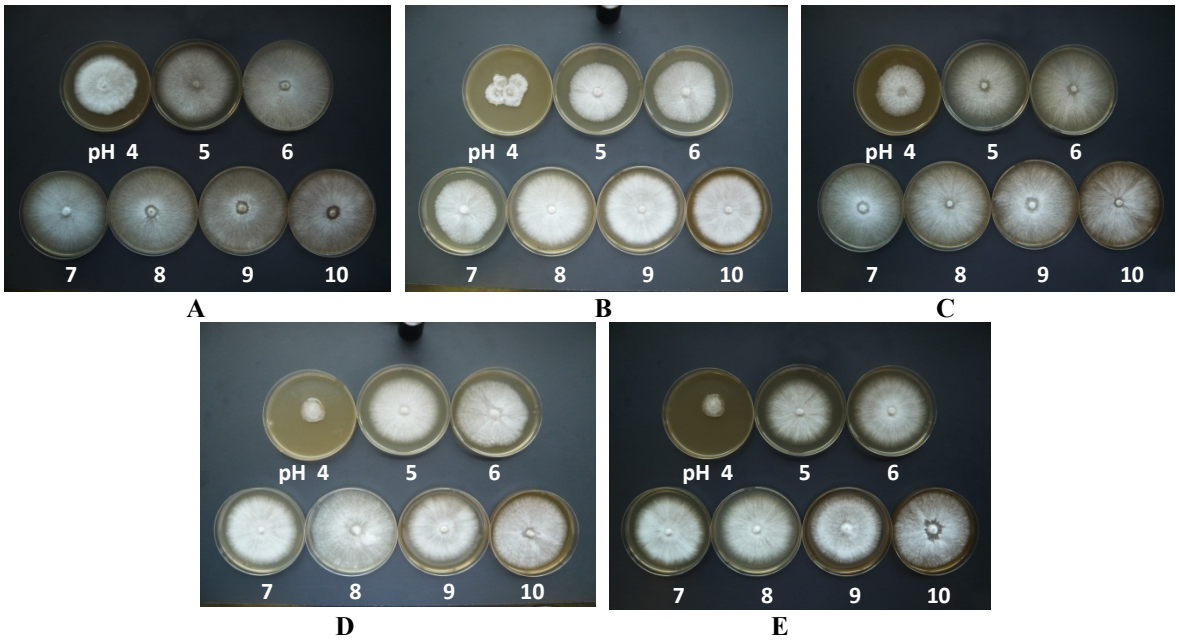

Figure 1. Mycelial growth of all strains of Hed Taen Rad on malt extract agar at various pH levels after incubation for 12 days at 30°C: A DOA-1, B DOA-4, C DOA-7, D DOA-10, E DOA

Morphological characterization of basidiomata

Morphological characters is still useful for preliminary evaluation because it is fast, simple, and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions (Beyene et al. 2005). The qualitative characteristics of the basidiocarps of all five strains of Hed Taen Rad were examined. Fruiting bodies were broad shaped. The pileus diameter and stipe length of the five *M. crassa* isolates were divided into 2 groups. Group 1 included DOA, DOA-1, DOA-7 and DOA-10 with a relatively large round-shaped pileus at 5.58 to 11.70 cm diameter; the stipes were cylindrical, swollen at the base with length 6.83-13.67 cm and diameter 1.30-3.72 cm. Group 2 included DOA-4 with a convex, depressed center pileus at 5.81-8.95 cm diameter, the surface was pale cream with a short stipe from 8.7 to 11.8 cm. Basidiospores of the five isolates of Hed Taen Rad were hyaline and oval with length 5.0-7.5 and diameter 3.0-4.5 µm. Payapanon and Srijumpa (2008) found that spores of Hed Taen Rad were white and oval with length 8.3-10.5 and diameter 6.0-7.5 µm, while Pegler et al. (1998) reported that spores of *M. crassa* had thin walls and a smooth surface and varied in length from 5.0-6.5 µm with diameter 3.7-4.5 µm. Basidia of this mushroom were clavate shaped with length 23.5-35.0 µm and diameter 7.0-8.0 µm with four sterigmata (Figure 2) and no cystidia. Thus, it was concluded that the morphological characterization of basidiomata of all five strains: DOA, DOA-1, DOA-4, DOA-7 and DOA-10 were closely similar to *M. crassa* (Berk.) Pegler & Lodge (Table 1).

Molecular identification of *M. crassa*

The 20 ITS sequences were deposited in the GenBank database (htpp://www.ncbi.nlm.nih.gov/) with the accession numbers shown in Table 2. The designations were based on morphological species concepts.

The species designations were based on the morphological species concepts. Amplification of the ITS1 and ITS2 regions from all isolates of *Macrocybe* (*Tricholoma*) using the primers ITS5 and ITS4 resulted in a 545-778 bp product. ITS sequences of DOA and DOA-10 strains isolated from a mycelium of Hed Taen Rad showed the highest levels of similarity (99%, identity = 585/585 nt and identity = 582/586 nt) with a particular ITS1-5.8S-ITS2 sequence of *M. crassa* (accession number LC006057.1). ITS sequences of DOA-1 and DOA-7 showed a significantly high level of ITS similarity (98.61% and 98.43%) from *M. crassa*. However, the ITS sequence obtained from DOA-4 strain showed only 86.71% similarity, with a particular ITS1-5.8S-ITS2 sequence. Molecular analysis of the ITS region showed a well-supported clade with 99% bootstrap support including all unknown species analyzed. The phylogenetic position from the PAUP analysis presented clusters of the sample of Hed Taen Rad (*Macrocybe* spp.) collected in Thailand into two major groups (Figure 3). The sequences from the four strains; DOA, DOA-1, DOA-7 and DOA-10 were identified as a *T. giganteum* cluster in one clade with 99% support, along with one sequence from the GenBank, *T. giganteum*. The strain DOA-4 was identified as *T. giganteum* with a 57% support. From this study, the dendrogram revealed relationships between strains of *Macrocybe* (*Tricholoma*) spp. The molecular identification of isolates DOA and DOA-10 presented a more intimate relationship with strains DOA-1 and DOA-7. The cultivation of this mushroom was originally described in 1958 and identified by The Royal Botanic Gardens, Kew, England which classified it as *T. crassum* (Berk) Sacc. (Chaiwongkeit 1985). By comparing the nucleotide sequences from the ITS region of the nuclear ribosomal DNA with the GenBank database for the five strains collected in Thailand, results showed that four strains were *T. giganteum* species, i.e. DOA, DOA-1, DOA-7 and DOA-10, and the other species (DOA-4 strain) may not be the same type. However, it remained identified as *T. giganteum.*

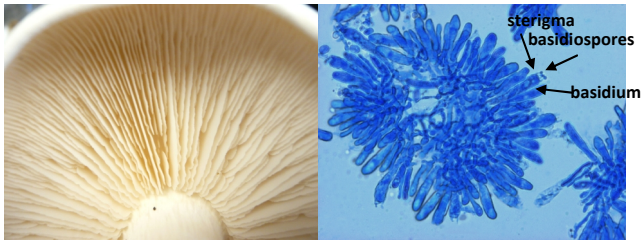

Figure 2. Gills (*left*), basidia, sterigma and basidiospores (400x) (*right*) of Hed Taen Rad

Table 2. Data and GenBank accession numbers of the sequences used for molecular analyses

Species	Culture/ herbarium number	Origin	GenBank accession number
T. giganteum	-	China	EU051917.1
T. giganteum	-	India	JN192443.1
T. giganteum	-	India	JN006792.1
T. giganteum	RCK2012	India	JQ957908.1
T. giganteum	CBE	India	HM120872.1
T. giganteum	SCAU 2	China	JX068526.1
T. matsutake	-	Japan	AF204868.1
T. imbricatum	TIK1-IX-09	Montenegro	JQ685731.1
T. imbricatum	TIK2-X-10	Montenegro	JQ685732.1
T. imbricatum	KMS296	USA	AF377242.1
T. batschii	TBG-XI-09	Montenegro	JQ685729.1
T. batschii	TBK-X-06	Montenegro	JQ685730.1
T. portentosum	KMS304	USA	AF349686.1
T. portentosum	-	Canada	HQ650742.1
T. myomyces	SMI323	Canada	FJ845443.1
T. myomyces var. *cystidiotum*	KMS281	USA	AF349699.1
M. (Tricholoma) spp.	DOA	Thailand	LC029415.1
M. (Tricholoma) spp.	DOA-1	Thailand	LC029416.1
M. (Tricholoma) spp.	DOA-4	Thailand	LC029418.1
M. (Tricholoma) spp.	DOA-7	Thailand	LC029417.1
M. (Tricholoma) spp.	DOA-10	Thailand	LC006057.1
Boletus edulis	YM6	China	EF646278.1
Boletus edulis	2044	Spain	HM579930.1
Pleurotus ostreatus	NW424	China	EU622250.1
Pleurotus ostreatus	NW446	China	EU622256.1

Table 1. Morphological characteristics of basidiomata of the five wild Thai isolates of the tropical mushroom Hed Taen Rad (*M. crassa*) compared with strains of *M. crassa* (Berk.) Pegler & Lodge and *Macrocybe gigantea* (Massee) Pegler & Lodge as the reference strain

Morphological characterization	**DOA**	**DOA-1**	**DOA-4**	**DOA-7**	**DOA-10**	***Macrocybe crassa* (Berk.) Pegler & Lodge***	***Macrocybe gigantea* (Massee) Pegler & Lodge***
Pileus	6.14-9.3 cm diameter, convex, surface pale cream, darker at center, smooth, drying minutely cracked, margin involute, weakly crenate	5.58-7.83 cm diameter, convex becoming slightly concave, surface pale cream, darker at center, smooth, drying minutely cracked, margin involute	5.81-8.95 cm diameter, convex and depressed center, surface pale cream, darker at center, smooth, drying minutely cracked, margin lobed, often cracking	6.0-8.50 cm diameter, convex, surface pale cream, smooth, margin often slightly wavy, thick	6.06-11.70 cm diameter, convex or almost applanate, a similar dish upside (width is greater than height), a half circle, incurved margin, thin, surface pale cream, darker at center, smooth, drying minutely cracked	14-24 cm diameter, convex to obtusely umbonate or almost applanate, finally becoming slightly depressed; surface pale cream, yellowish brown to grayish brown, smooth, drying minutely cracked and sometimes splitting radially; margin involute, weakly crenate	15-18 × 6 cm, cylindrical, often elongate, solid finally fistulose; surface concolorous with pileus, fibrillose-striate
Lamellae	Adnexed, pale cream, 4-7 mm broad, crowded	Adnexed, pale cream, 4-7 mm broad, crowded	Adnexed, pale cream, 3-7 mm broad, crowded	Adnexed, pale cream, 4-9 mm broad, crowded	Adnexed, pale cream to white, 4-9 mm broad, crowded	Adnexed to sinuate, white broad, crowded, with lamellulae of two lengths.	Emarginate, sinuate, straw yellow, ventricose, densely crowded, with lamellulae of four lengths.
Stipe	7.06-11.40 × 1.71-3.17 cm, cylindrical, swollen at base; solid; surface off- white, with brown fibrillose streaks	6.83-12.10 × 1.77-2.92 cm, cylindrical, swollen at base, solid, surface off- white, with brown fibrillose streaks	Short with 6.93-10.20 × 1.71-2.34 cm, cylindrical, swollen at base, solid, surface concolorous with pileus, with brown fibrillose streaks	6.90-13.67 × 1.30-3.72 cm wide, cylindrical, enlarged toward base, surface white ,brown fibrillose streaks	6.90-13.67 × 1.30-3.72 cm, cylindrical, swollen at base, surface off- white, with brown fibrillose streaks	15-25 × 1.4-5 cm, cylindrical, swollen at base; solid then fistulose; surface off-white, with brown fibrillose streaks	15-18 × 6 cm, cylindrical, often elongate, solid finally fistulose; surface concolorous with pileus, fibrillose-striate

Context	Thick at disc, white, firm, consisting of thin-walled hyphae, inflated, with clamp connections	Thick at disc, white, firm, consisting of thin-walled hyphae, inflated, with clamp connections	Thick at disc, white, firm, consisting of thin-walled hyphae, inflated, with clamp connections	Thick at disc, white, firm, consisting of thin-walled hyphae, inflated, with clamp connections, taste slightly bitter	Thick at disc, white, firm, consisting of thin-walled hyphae, inflated, with clamp connections	3.5 cm thick at disc, white, firm; consisting of thin-walled hyphae, 2-6 µm diameter, with clamp connections, taste slightly bitter	3 cm thick at disc, white, firm; consisting of thin-walled hyphae, 2-8 µm diameter, inflated to 25 µm diameter, with clamp connections; odor recalling 'brewer's grains'
Spores	Spore deposit pale cream. Spores 6.5-7.0 × 3.0-4.5 µm, ovoid, hyaline, inamyloid, thin-walled, smooth	Spore deposit pale cream. Spores 6-6.5 × 3.0-4.0 µm, ovoid, hyaline, inamyloid, thin-walled, smooth	Spore deposit pale cream. Spores 6-7.5 × 3.0-4.0 µm, ovoid, hyaline, thin-walled, smooth	Spore deposit pale cream. Spores 5.0-6.5 × 3.5-4.0 µm, ovoid, hyaline, thin-walled, smooth	Spore deposit pale cream. Spores 6.0-7.0 × 4-4.5 µm, ovoid, hyaline, thin-walled, smooth	Spore deposit pale cream. Spores 5.0-6.5 × 3.7-4.5 (5.60 ± 0.15 × 4.20 ± 0.32) µm, ovoid, hyaline, thin-walled, smooth.	Spore deposit white. Spores 5.7-7.5 × 4.0-5.3 (6.70 ± 0.90 × 4.60 ± 0.38) µm, ovoid to short ellipsoid, hyaline, inamyloid, thin-walled
Basidia	23.5-29 × 7-8 µm, clavate, bearing four sterigmata. Hymenial cystidia none	23.5-29 × 7-8 µm, clavate, bearing four sterigmata. Hymenial cystidia none	23.5-29 × 7-8 µm, clavate, bearing four sterigmata. Hymenial cystidia none	26-35 × 7-8 µm, clavate, bearing four sterigmata. Hymenial cystidia none	24-31 × 7-8 µm, clavate, four sterigmata,cystidia none	25-30 × 7-8 µm, clavate, bearing four sterigmata	25-37 × 5-8 µm, narrowly clavate to subcylindrical, bearing four sterigmata; with basal clamp connection
Hymenophoral trama	Regular, with hyphae 3-5 µm diameter	Regular, with hyphae 3-5 µm diameter	Regular, with hyphae 2.5-4 µm diameter	Regular, with hyphae 2.5-4.5 µm diameter	Regular, with hyphae 3-5 µm diameter	Regular, with hyphae 3-5 µm diameter	Regular, of parallel, thin-walled hyphae, 2-5 µm diameter, with clamp connections

Note: *) Pegler et al.(1998)

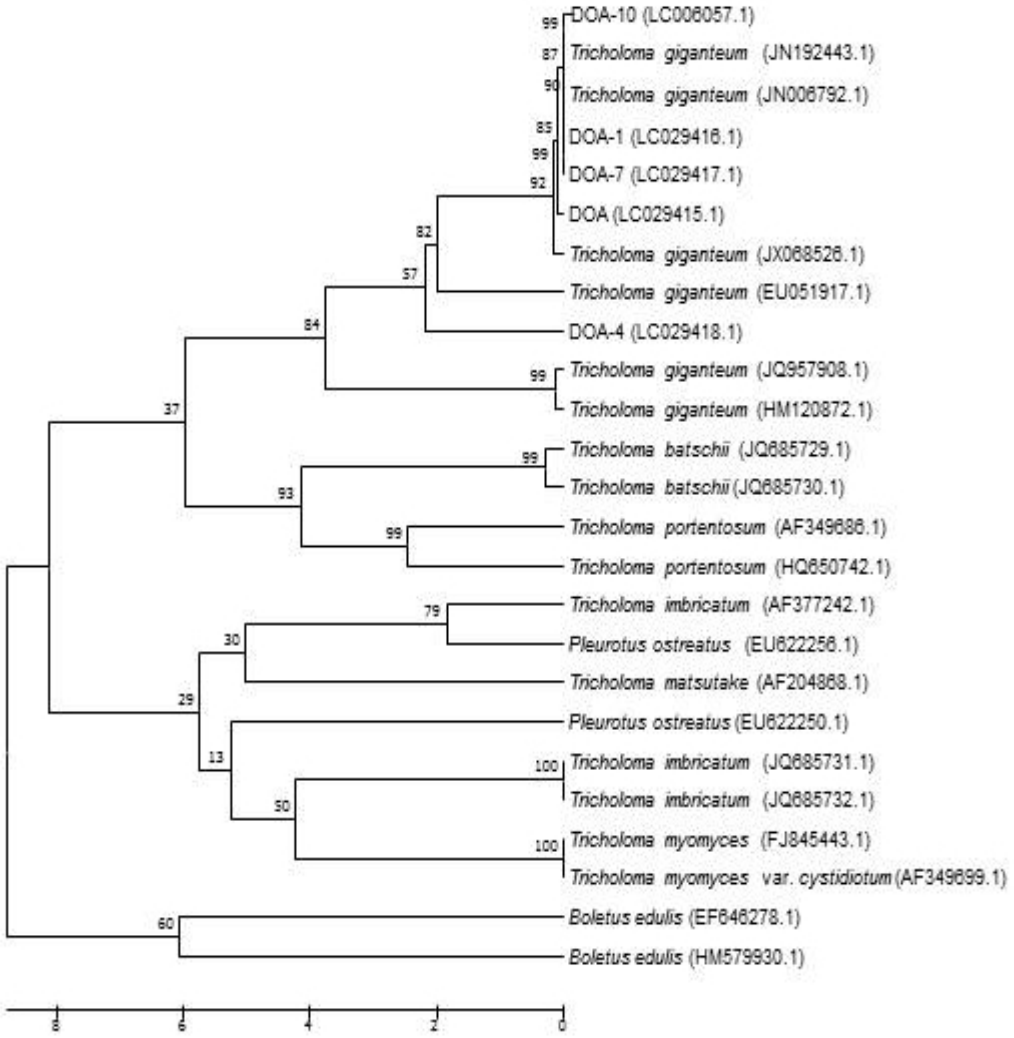

Figure 3. Phylogenetic relationships of Hed Taen Rad (*Macrocybe* spp.) inferred from the ITS sequences of 20 equally parsimonious trees. *P. ostreatus* and *Boletus edulis* were used as an outgroup. Numbers above the branches indicate the percentage at which a given branch was supported in 1000 bootstrap replications. Branches within a vertical line represent the same family.

The results implied that the nucleotide sequences of *M. crassa* and *T. giganteum* were very similar. Unfortunately, the nucleotide sequences of *M. crassa* or *T. crassum* have not yet been recorded in the GenBank database. Only four nucleotide sequences of *Macrocybe* sp. have been deposited with 2,338 for *Tricholoma* sp. (htpp://www.ncbi.nlm.nih.gov/). To date, limited ITS rDNA sequences from *M. crassa* exist in public databases, with few covering the entire ITS length. Therefore, the identification of five strains of Hed Taen Rad using only molecular methods could be erroneous. However, further differentiation using molecular markers was recommended.

This taxonomic study examined the morphological characteristics and molecular identification of the mycelia and basidiomata from five isolates of Hed Taen Rad: DOA, DOA-1, DOA-4, DOA-7 and DOA-10. Results showed that they grew well on MEA at pH 8-10, with thick mycelial density at 20-30°C obtaining an 8.50 cm diameter colony after 9 days.

Comparisons of the morphological characteristics of all five strains of this mushroom to *M. gigantea* (Massee) Pegler & Lodge, or *T. giganteum* Massee in Bull. Misc. Inf. Kew 1912: 254. 1912 were clearly different as the stipes were cylindrical, and swollen at the base while *M. gigantea* had cylindrical-shaped, often elongate and not inflated at the base. Moreover, they presented clavate-shaped basidia with morphological characterization of the basidiomata very similar to *M. crassa* (Berk.) Pegler & Lodge or *T. crassum* (Berk.) Sacc., Syll. Fung. 5: 109 (Pegler et al. 1998) (Table 1). Thus, all five strains of Hed Taen Rad mushroom could be *M. crassa*. On the other hand, from molecular analysis of all five isolates were identified as a *T. giganteum* cluster with 99% support and 57% support. From this experiment, may be interpreted incorrectly since

the nucleotide sequences of *M. crassa* or *T. crassum* have not yet been recorded in the GenBank database and ITS regions only one position may not be suitable to be used in the identification. Thus, all five strains of these mushrooms were successfully characterized using morphological character as *M. crassa*. This study has contributed knowledge for precise identification and provided additional data to the major database of GenBank to prevent future misidentification of this mushroom species. Species of fungi with ecological proximity but different geographical origins can be classified through morphological and molecular markers. This study demonstrated that morphological evaluation proved useful to identify relationships among species of a wild Thai isolate of the tropical mushroom Hed Taen Rad (*M. crassa*).

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