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# *Auricularia thailandica sp. nov. (Auriculariaceae, Auriculariales)* a widely distributed species from Southeastern Asia

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# Abstract

*Auricularia* is an important genus among the jelly fungi due to its popular consumption and medicinal properties. A new species of *Auricularia*, *A. thailandica* is described from fresh collections made from the Philippines, Thailand and Southern China based on morphological and molecular characters. *Auricularia thailandica* differs from other species by having short and loosely arranged abhymenial hairs on the basidiomata and in the different size of the zones in a cross section of the basidiomata. The species is found to be widely distributed in Southeastern Asia. Phylogenetic relationships were inferred based on the nuclear ribosomal internal transcribed spacer (ITS) region. The new species is introduced with full description and illustrations.

Key words: Jelly fungi, morphology, phylogeny, taxonomy

# Introduction

*Auricularia* Bull. is a genus of jelly fungi in the family *Auriculariaceae* Fr., typified by *Auricularia mesenterica* (Dicks.: Fr.) Pers. Species of *Auricularia* are distributed in tropical, subtropical and temperate regions (Lowy, 1952). Most *Auricularia* species are edible and *Auricularia auricula-judae* (Bull.: Fr.) Queil. and *A. polytricha* (Mont.) Sacc. are widely produced commercially (Wu *et al.*, 2014a; Yan *et al.*, 2004).

As the macroscopic features of *Auricularia* vary with the age of the specimen, exposure to light, availability of moisture and other environmental factors; the current morphological classification of *Auricularia* is based on internal stratification of different layers and abhymenial hairs on the basidiomata (Kobayashi, 1981; Lowy, 1951). Ten species were described worldwide by Lowy (1952). Later on, mating studies (Duncan & MacDonald, 1967) and differences of spore sizes (Parmasto & Parmasto, 1987) were used to classify species. Fifteen species and five variants of *Auricularia* were monographed by Kobayashi (1981). A recent study estimated that this genus comprises 10–15 species throughout the world (Looney *et al.*, 2013), while Kirk *et al.* (2008) estimated there are eight species worldwide.

Phylogenetic analysis of ITS shows that *Auricularia* is a monophyletic genus (Weiß & Oberwinkler, 2001). ITS analysis has separated *A. auricula-judae*, *A. polytricha* and *A. fuscosuccinea* into three well-supported clades (Montoya-Alvarez *et al.*, 2011). Further analyses of ITS with a large sample size revealed nine species of *Auricularia* with relevant morphological and ecological characters (Looney *et al.*, 2013). In addition, the analysis of *rpb2* showed similar groupings as the ITS sequence results, but with a higher bootstrap support (Looney *et al.*, 2013).

According to the literature, 15 species of *Auricularia* have been recorded in China based on morphology. However, due to the invalid nomenclature of *A. reticulata* L.J. Li and misidentification of *A. rugosissima*, both species cannot be considered as members of the genus (Wu *et al.*, 2014a). Molecular analyses have been used for species delineation. RFLP and RAPD techniques classified eight *Auricularia* species in China (Yan *et al.*, 2002; Yan *et al.*, 1999). Wen *et al.* (2005) identified three species of *Auricularia* using ERIC analysis. *Auricularia auricula*, *A. delicata*, *A. fuscosuccinea* and *A. polytricha* were confirmed to occur in China based on ITS sequence analysis (Wang *et al.*, 2013). Overall, phylogenetic studies of this genus in China are however, still at the initial stage (Wu *et al.*, 2014a).

Of the ten *Auricularia* species re-described by Lowy (1952), eight were recorded by Lalap (1981) from the Philippines (Musngi *et al.*, 2005). One species of *Auriculariaceae* was reported in a study on the mushroom diversity at Mt. Malinao, Albay (Daep & Cajuday, 2003). Four species of *Auricularia (A. auricula, A. fuscossucinea, A. polytricha* and *A. tenuis*), were collected in the University grounds of the Central Luzon State University (Musngi *et al.*, 2005) and *A. fuscossucinea* was found at Puncan, Carranglan, Nueva Ecija (Sibounnavong *et al.*, 2008). *Auricularia mesenterica* and an unknown *Auricularia* species were found with three previously recorded species from central Luzon in Philippines (De Leon *et al.*, 2013). All the previous studies of *Auricularia* in Philippines were based on morphology, while this is the first study using morphology and a molecular phylogeny.

*Auricularia* species (locally called Hed Hoo Noo) are popular edible mushrooms in Thailand (Jones *et al.*, 1994; Klomklung *et al.*, 2012). However, there is no scientific literature on *Auricularia* in Thailand, except the field guides that report *A. delicata, A. fuscosuccinea, A. mesenterica* and *A. polytricha* (Chandrasrikul *et al.*, 2008; Ruksawong & Flegel, 2001). There have been very few studies on the genus *Auricularia* in Thailand and this study is the first report based using both morphology and molecular phylogeny to define species.

In this study, we introduce a novel species of *Auricularia* from three Southeast Asian countries: China, Philippines and Thailand. The morphology-based classification of Lowy (1951) and ITS sequence data were used to define species boundaries in the genus.

# **Materials and Methods**

# Sample collection

Fruiting bodies were collected on decaying wood from Mengsong in China, Unisan, Quezon in Philippines and Chiang Mai and Chiang Rai provinces in Thailand during the rainy seasons of 2012–2013. The specimens were hot air dried (50°C) and sealed in Ziplock plastic bags containing dehydrated silica gel as a desiccant to control humidity. All herbarium specimens are deposited in the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Duplicate specimens are deposited in Kunming Institute of Botany, Chinese Academy of Sciences (KUN), Kunming, Yunnan, China.

# Morphological character examination

Macro-morphological characters were described based on the fresh material, and photographed. Colour notations in the descriptions were from Kornerup & Wanscher (1978). Microscopic characters were studied from free-hand sections of the dried material mounted in distilled water at a magnification up to  $1000 \times$  with a Nikon Eclipse 80i (Nikon, Tokyo, Japan) microscope. Microphotography of the internal details was done under the same microscope with a Canon EOS 550D (Tokyo, Japan) camera mounted on top. Measurements were taken using Image framework (Tarosoft, v0.9.7). The spore shape quotient (Q = L/W) was calculated considering the mean value of the lengths and widths of 30 basidiospores.

# DNA extraction, PCR and sequencing

Dried basidiomata were ground using a porcelain mortar and pestle. DNA was extracted from the ground product using an E.Z.N.A.<sup>®</sup> Forensic DNA kit, D3591–01, (Omega Bio-Tek, Norcross, GA). The nuclear ribosomal ITS1-5.8S-ITS2 barcode region was amplified using standard primer pairs ITS4/ITS5 following the polymerase chain reaction protocol of White *et al.* (1990). Sequencing was performed on ABI 3730 XL DNA analyser (Applied Biosystems) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China and BiK-F laboratory, Frankfurt am Main, Germany.

# Phylogenetic analyses

The ITS sequences derived from this study (Table 1, in bold) plus those retrieved from GenBank were aligned using MAFFT online server (Katoh & Standley, 2013) and manually adjusted using Bioedit v7.2.5 (Hall, 1999). Maximum Likelihood (ML) analysis was performed by raxmlGUI v1.31 (Silvestro & Michalak, 2012) using rapid bootstrap analysis with 1000 replicates in GTR model. *Exidia recisa* (Ditmar) Fr., and *Exidiopsis sp.* were used as outgroup taxa

(Weiß & Oberwinkler, 2001). Maximum Parsimony (MP) analysis was performed with PAUP v. 4.0b10 (Swofford, 2003). Trees were inferred by using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. The maximum number of retained trees was limited to 10,000, branches of zero length were collapsed and all multiple equally most parsimonious trees were saved. A most suitable model for the Bayesian analysis was selected using MrModeltest v. 2.2 (Nylander, 2004). The Bayesian analyses (MrBayes v. 3.2; Ronquist *et al.*, 2012) of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run from random trees for 100,000,000 generations and sampled every 1,000 generations. The temperature value was lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01. We consider bootstrap support >70 as strong support, between 50 and 70 as moderate support and below 50 as poor support.

| Species               | Herberium  | Collection   | GenBank accession | Locality                   |
|-----------------------|------------|--------------|-------------------|----------------------------|
|                       |            |              | ITS               |                            |
| Auricularia americana | TENN049666 | TFB2897      | JX065152          | North Carolina, USA        |
| A. americana          | TENN051203 | TFB4651      | JX065151          | Tennessee, USA             |
| 4. americana          | TENN061466 | TFB13202     | JX065146          | Tennessee, USA             |
| 4. americana          | TENN067030 | BPL116       | JX065163          | Tennessee, USA             |
| 4. americana          | TENN067029 | BPL112       | JX065166          | Tennessee, USA             |
| 4. americana          | TENN052403 | TFB5612      | JX065154          | Idaho, USA                 |
| 4. americana          |            | PBM2295      | DQ200918          | Oregon, USA                |
| 4. auricula-judae     | MFLU130394 |              | KR336695          | Kassel, Germany            |
| 4. auricula-judae     |            | MW446        | DQ520099          | Germany                    |
| 4. auricula-judae     |            | MFUAB38      | KR336696          | Rome, Italy                |
| 1. auricula-judae     | TENN050632 | TFB4296      | JX065174          | Switzerland                |
| 4. auricula-judae     |            |              | HQ388355          | China                      |
| 4. auricula-judae     |            |              | FJ478123          | China                      |
| 4. auricula-judae     |            | AFM21        | AB615232          | Kochi, Japan               |
| 1. auricula-judae     |            |              | HQ388358          | China                      |
| 4. cornea             | TENN066990 | PBM3754      | JX065164          | Queensland, Australia      |
| 1. cornea             |            |              | HM448454          | China                      |
| 4. cornea             |            |              | HM448462          | China                      |
| 4. cornea             |            | MFUAB36      | KR336702          | Zhaoquing, China           |
| 4. cornea             | PDD92640   |              | KR336699          | Wairarapa, NewZealand      |
| 4. cornea             | PDD97684   |              | KR336700          | Wellington, New Zealand    |
| 4. cornea             | PDD103780  |              | KR336701          | Bay of Plenty, New Zealand |
| 4. cornea             | TENN049019 | TFB3470      | JX065149          | Puerto Rico                |
| 4. delicata           | TENN067025 | CNSBlitz0012 | JX065169          | Queensland, Australia      |
| 4. delicata           | TENN067026 | CNSBlitz0050 | JX065168          | Queensland, Australia      |
| 4. delicata           | TENN067027 | CNSBlitz0093 | JX065165          | Queensland, Australia      |
| 1. delicata           | TENN067028 | CNSBlitz0098 | JX065171          | Queensland, Australia      |
| 1. delicata           |            | USJ54470     | AF291269          | Costa Rica                 |
| 1. delicata           | TENN028734 | TENN28734    | JX065159          | Loreto, Peru               |
| 4. delicata           | TENN017797 | AJS5896      | JX065162          | San Luis Potosiì, Mexico   |
| 1. delicata           | TENN016963 | AJS1304      | JX065158          | Veracruz, Mexico           |
| 4. fuscosuccinea      | TENN058951 | TFB10743     | JX065141          | Buenos Aires, Argentina    |
| 4. fuscosuccinea      | TENN056263 | TFB9503      | JX065157          | Louisiana, USA             |
| 4. fuscosuccinea      | TENN059094 | TFB11289     | JX065153          | Misiones, Argentina        |
| 4. fuscosuccinea      | TENN019800 | LRH19800     | JX065138          | Tamaulipas, Mexico         |
| 4. fuscosuccinea      | TENN063200 | KF09         | JX065173          | Tennessee, USA             |
| 1. heimuer            |            | Cui7100      | KM396796          | Jilin, China               |
| 1. heimuer            |            | Dai13647     | KM396790          | Jilin, China               |
| 1. heimuer            |            | Dai13765     | KM396793          | Heilongjiang, China        |
| 4. heimuer            | LE296423   |              | KJ698422          | Russia                     |
| 4. mesenterica        | MFLU130393 |              | KR336697          | Edersee, Germany           |
| A. mesenterica        |            | FO25132      | AF291271          | Germany                    |
| A. mesenterica        |            | MFUAB39      | KR336698          | Rome, Italy                |
| A. minor              | LE 296424  |              | KJ698434          | Russia                     |

**TABLE 1**. Sequences used in the phylogenetic analyses. Newly produced sequences are in bold.

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AURICULARIA THAILANDICA SP. NOV.

#### TABLE 1. (Continued)

| Species        | Herberium  | Collection  | GenBank accession | Locality                    |
|----------------|------------|-------------|-------------------|-----------------------------|
|                |            |             | ITS               |                             |
| A. nigricans   | TENN056825 | TFB4405     | JX065172          | Louisiana, USA              |
| A. nigricans   | TENN059115 | TFB11410    | JX065176          | Misiones, Argentina         |
| A. nigricans   | WTU        | JMB04010803 | JX065167          | San Joseì, Costa Rica       |
| A. scissa      | FH00301771 | F64         | JX065175          | Florida, USA                |
| A. scissa      | TENN059729 | TFB11193    | JX065160          | La Vega, Dominican Republic |
| A. subglabra   | TENN057615 | TFB10046    | JX524199          | Alajuela, Costa Rica        |
| A. subglabra   | TENN053855 | TFB7868     | JX065142          | Puntarenas, Costa Rica      |
| A. subglabra   | TENN058607 | TFB10499    | JX065155          | Puntarenas, Costa Rica      |
| A. subglabra   | TENN058100 | TFB10405    | JX065161          | San Joseì, Costa Rica       |
| A. thailandica | MFLU130396 |             | KR336690          | Chiang Mai, Thailand        |
| A. thailandica | MFLU130399 |             | KR336691          | Chiang Rai, Thailand        |
| A. thailandica | MFLU130410 |             | KR336693          | Chiang Rai, Thailand        |
| A. thailandica | MFLU130411 |             | KR336694          | Mengsong, China             |
| A. thailandica | MFLU130417 |             | KR336692          | Quezon, Philippine          |
| A. villosula   |            | Dai 13453   | KM396813          | Jiangxi, China              |
| A. villosula   |            | Dai 13652   | KM396814          | Chongqing, China            |
| A. villosula   |            | HMAS 130446 | KM396817          | Tibet, China                |
| A. villosula   | LE 296422  |             | KJ698418          | Russia                      |
| Exidia recisa  |            | MW315       | AF291276          | Sweden                      |
| Exidiopsis sp. |            | FO46291     | AF291282          | Germany                     |

#### Results

#### Phylogeny

The final ITS dataset comprised 63 sequences of *Auricularia*, with *Exidia recisa* and *Exidiopsis sp.* as outgroup taxa. The final alignment comprised 529 characters, of which 382 were constant, 25 were parsimony-uninformative and 122 were parsimony-informative characters. The ML tree is shown in Fig. 1. In total 13 species defined by morphological characters were included in the phylogenetic analysis.

Ten distinct clades in the ITS tree were strongly supported and defined the following species: *A. auricula-judae* (100/100/99), *A. cornea* (82/100/90), *A. fuscosuccinea* (93/94/88), *A. heimuer* (90/100/86), *A. mesenterica* (100/100/100), *A. nigricans* (89/90/77), *A. scissa* (98/99/79), *A. subglabra* (100/100/99) and *A. villosula* (99/100/100), with a new species labeled as *A. thailandica* (100/100/100) introduced in this study. The collections labeled as *A. delicata* and *A. americana* are polyphyletic or paraphyletic.

#### Taxonomy

*Auricularia thailandica* Bandara & K.D. Hyde, *sp. nov.* (Fig. 2) Index Fungorum Number: IF550992, *Facesoffungi number*: FoF 00467.

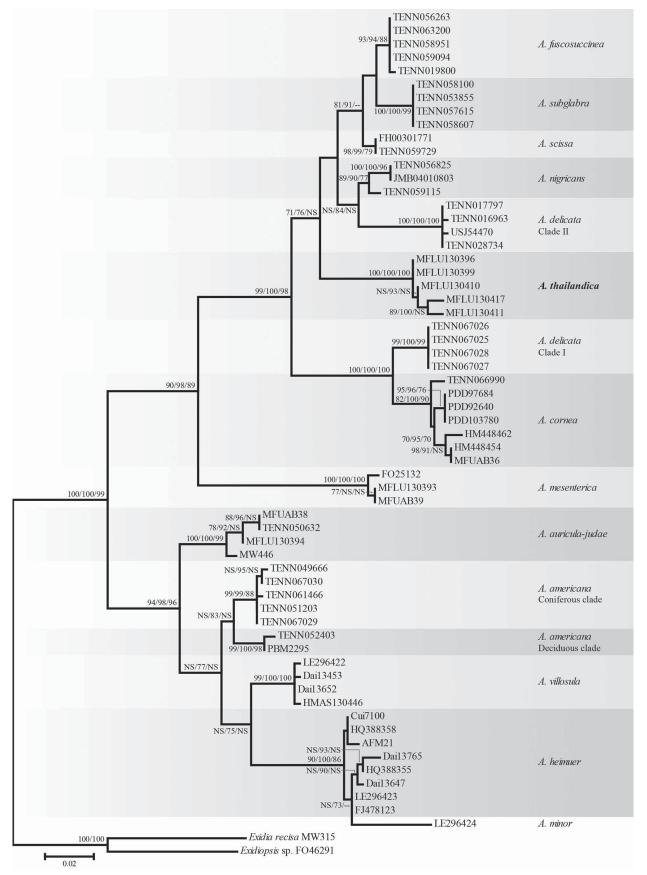
Type:—THAILAND. Chiang Mai: Bahn Pa Dheng, Mushroom Research Center, dead wood, 14 August 2013, S.C. Karunarathne K2013117 (MFLU 130410, holotype).

Etymology:-The species epithet "thailandica" refers to the country from where the holotype was collected.

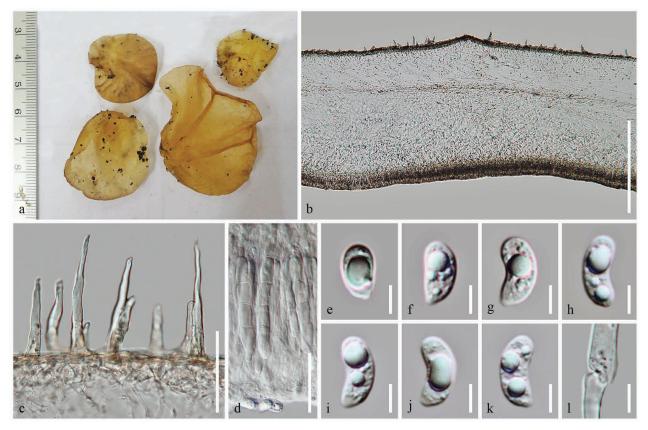
Basidiome:—2–5 cm diam., short stalks, orbicular to cupulate to auriculiform, semi-transparent, brownish orange, 5C5, ridges and veins seen on abhymenial surface, individual hairs clearly distinguishable, hairs not dense, margins darker brown, 6E8.

Internal features: thickness 340–700  $\mu$ m; medulla present; abhymenial hairs scattered, non-gregarious, hyaline, acute tip, thin or thick walled, 0.9–3.7  $\mu$ m, clear lumen, septa observed, hair bases 5–11.4  $\mu$ m wide, hair bases pigmented, light brown, plasmatic; clamp connections present; zona pilosa  $\leq$ 80  $\mu$ m; zona compacta 23–46  $\mu$ m; zona subcompacta superioris 10–29  $\mu$ m; zona laxa superioris 35–181  $\mu$ m; medulla 31–98  $\mu$ m; zona laxa inferioris 98–307  $\mu$ m; zona subcompacta inferioris 21–78  $\mu$ m; hymenium 44–78  $\mu$ m; basidia 34.67–48.41  $\times$  2.73–6.31  $\mu$ m, cylindrical,

tapered or blunt ends, transversely 3-septate, sterigmata observed; spores smooth walled, allantoid,  $9.47-13.10 \times 4.45-5.66 \mu m$ , Q = 1.80–2.80, with one or two large guttules.



**FIGURE 1.** The phylogram inferred from a Maximum Likelihood analysis of ITS sequences of *Auricularia*. Species names are followed by the herbarium code or collection code or GenBank accession number. RAxML/ Bayesian posterior probabilities / MP bootstrap values  $\geq$ 70 % are displayed above or below each node. The tree is rooted with *Exidia recisa* (MW315) and *Exidiopsis sp.* (FO46291).



**FIGURE 2.** *Auricularia thailandica* (MFLU 130410, holotype). a. Basidiocarps. b. Cross-section of the fruitbody. c. Abhymenial hairs. d. Close-up of hymenial layer. e–k. Basidiospores. l. Clamp-connection of a hypha. Scale bars:  $b=500 \mu m$ ,  $c=50 \mu m$ ,  $d=25 \mu m$ ,  $e-l=5 \mu m$ . (Photos by Asanka R. Bandara).

Collections examined:—CHINA. Yunnan: Mengsong, Plot-145, on dead wood, 23 May 2012, S.C. Karunarathne MS09 (MFLU 130411); THAILAND. Chiang Mai: Bahn Pa Dheng, Mushroom Research Center, dead wood, 12 June 2013, A.B. Bandara AB201323 (MFLU 130396); THAILAND. Chiang Rai: Doi Mae Salong, decaying tree trunk, June 2013, A.B. Bandara AB201326 (MFLU 130399); PHILIPPINES. Quezon: Unisan, on deadwood, March 2012, P. Alva PA30 (MFLU 130417).

Note:—*A. thailandica* can be distinguished from other species of *Auricularia* that have short and loosely arranged abhymenial hairs of the basidiomata. Among the species which have a medulla, the size of the zona pilosa of *A. thailandica* is similar to *A. fibrillifera*, *A. fuscosuccinea*, *A. minor*, *A. scissa* and *A. subglabra* (Table 2). The abhymenial hairs of *A. fuscosuccinea* and *A. scissa* are gregarious and tufted (Looney *et al.*, 2013). Although *A. minor* is characterized by short abhymenial hairs the basidiomata are smaller (less than 2 cm) (Malysheva & Bulakh, 2014) than *A. thailandica*. *A. fibrillifera* is distinct from *A. thailandica* in having thick-walled, acute, free or partly fasciculate hairs and thinner zones (Kobayashi, 1981). *A. subglabra* has solitary, infrequent hairs (Looney *et al.*, 2013). *A. subglabra* is distinguished from *A. thailandica* by the lack of abhymenial hairs, with only a few short hairs present at irregular intervals throughout the zona pilosa.

# Discussion

The use of the internal zone of the basidiomata has been used as a taxonomic criterion for defining *Auricularia* species. The widths of zones are however, not always stable among specimens of the same species (Table 2; e.g. *A. cornea*, *A. fuscosuccinea* and *A. polytricha*). The thickness of basidioma zones of *A. thailandica* in cross section randomly overlap with those of certain other species (Table 2). The sizes of zones of *A. thailandica* in cross section are similar to certain corresponding zones of *A. tenuis* but not similar with all zones (Lowy, 1952; Wong & Wells, 1987). However, Wong & Wells (1987) synonymized *A. tenuis* to *A. cornea* based on interfertility and the presence of a double medulla in both taxa.

| e=entipsoid, T=Lustronth, 0=Forud, 175, 175, 175, 175, 175, 175, 175, 175   | compacta, zoo-zona  | subcompaci    | a superiorits, a | ZLO-ZUIIA I | axa superior | lls, M-IIIcuu<br>Larrach: 1001 | Id, ZLI-ZUIR  |              | IIS, ZAI-ZUII | a subcompact | compacia, ZSS-Zona subcompacia superions, ZLS-Zona laxa superioris, M-medulla, ZLI-Zona laxa interioris, ZSI-Zona subcompacia interioris, H-nymenium, a-anantoid, ci-ciavate, cu- curved, | ianioia, ci-ciavaic, cu- cui veu,  |
|---|---------------------|---------------|------------------|-------------|--------------|--------------------------------|---------------|--------------|---------------|--------------|---|------------------------------------|
| Im         ZP $\mu$ m         ZC $\mu$ m         ZSS $\mu$ m         ZLS $\mu$ m         M $\mu$ m         ZLL $\mu$ m         ZSI $\mu$ m         H $\mu$ m           180-200         70-80         20-30         40-50         570-600         30-40         70-80         80-90           0.6         70-310         25         65         180-185         130         150         35-40         115-120           0.6         70-310         25         65         150-160         30-40         70-80         80-90           0.5         65-100         5-6.5         10-13         100         30-50         150         25         50-60           .8         60-80         25-35         10-15         140-150         30-50         150         25         50-60           .8         60-80         25-35         10-15         140-150         30-50         40         40           .8         60-80         25-35         90         100         40-150         25         50-60           .4         35-90         30-355         50-55         90         100         40         80           .8         60-80         25-324         41-86         40         40         40 | cy-cymurcal, e-em   | psoid, i–iusi | IOIIII, 0–0V01   | iu '(Luwy,  | 1722), (NU   | Dayasm, 190                    | 1), •(LUUIIEY | (c107,.10 19 |               |              |   |                                    |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | Species             | TH mm         | ZP µm            | ZC µm       | ZSS µm       | ZLS µm                         | M μm          | ZLI µm       | ZSI μm        | H µm         | Basidia µm  | Spores µm                          |
| $\begin{array}{llllllllllllllllllllllllllllllllllll$  | *Auricularia cornea | 0.8 - 1       | 180 - 200        | 70-80       | 20-30        | 40–50                          | 570-600       | 30-40        | 70–80         | 80–90        | $45-55 \times 4-5$ cl   | $14-16 \times 5-6a$                |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$  | †A. cornea          | 0.55 - 0.6    | 70–310           | 25          | 65           | 180 - 185                      | 130           | 150          | 35-40         | 115-120      | $100{-}110 \times 4{-}5$ cl   | $8-10 \times 4$ f/e                |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$  | •A. cornea          | 0.8 - 1       | 211-336          | 26–29       | 45-79        | 320-410                        | 131-135       | 119–223      | 87-143        | 93–99        | $52-69 \times 3.2-6$  | $13.5 - 17.5 \times 6 - 7.5 a$     |
| 0.35-0.5 $65-100$ $5-6.5$ $10-13$ $100$ $30-50$ $150$ $25$ $50-60$ <i>inea</i> $0.5-0.8$ $60-80$ $25-35$ $10-15$ $140-150$ $30-50$ $150-160$ $60-70$ $70-80$ <i>inea</i> $0.3-0.4$ $35-90$ $30-35$ $50-55$ $90$ $100$ $40$ $40$ <i>inea</i> $1-4$ $38-136$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ <i>nea</i> $1-4$ $865-897$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $0.65-0.8$ $30-80$ $8-9$ $40-45$ $300$ $75-80$ $220-230$ $30-355$ $70$ $1-4$ $865-897$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $1-1.5$ $450$ $32-710$ $32-76$ $55-224$ $41-86$ $1-1.5$ $560$ $32-736$  | *†A. emini          | 0.6 - 0.9     | 3–5 cm           | 60 - 70     | 40-50        | 150 - 160                      | 90-100        | 140 - 150    | 40-50         | 60-70        | $45-55 \times 4-5$ cl-cy  | $12-14 \times 4-5 a$               |
| inea $0.5 - 0.8$ $60 - 80$ $25 - 35$ $10 - 15$ $140 - 150$ $30 - 50$ $150 - 160$ $60 - 70$ $70 - 80$ inea $0.3 - 0.4$ $35 - 90$ $30 - 35$ $50 - 55$ $90$ $100$ $40$ $40$ inea $1 - 4$ $38 - 136$ $13 - 45$ $30 - 111$ $82 - 607$ $32 - 361$ $26 - 506$ $55 - 224$ $41 - 86$ $0.65 - 0.8$ $30 - 80$ $8 - 9$ $40 - 455$ $300$ $75 - 80$ $220 - 230$ $30 - 335$ $70$ $1 - 4$ $865 - 897$ $13 - 45$ $30 - 111$ $82 - 607$ $32 - 361$ $26 - 506$ $55 - 224$ $41 - 86$ $1 - 1.5$ $450$ $20 - 25$ $32 - 361$ $25 - 520$ $30 - 30$ $30 - 30$ $30 - 30$ $1 - 1.5$ $5600$ $40 - 45$ $60$ $37 - 380$ $55 - 276$ $40 - 45$ $65 - 70$ $80 - 90$ $1 - 1.5$ $5600$ $40 - 45$ $60$ $37 - 386$ $55 - 124$ $41 - 86$  | †A. fibrillifera    | 0.35 - 0.5    | 65 - 100         | 5-6.5       | 10 - 13      | 100                            | 30-50         | 150          | 25            | 50-60        | I   | $11-12 \times 4-5$ a               |
| inea $0.3-0.4$ $35-90$ $30-35$ $50-55$ $90$ $100$ $40$ $40$ inea $1-4$ $38-136$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $0.65-0.8$ $30-80$ $8-9$ $40-45$ $300$ $75-80$ $220-230$ $30-35$ $70$ $1-4$ $865-897$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $1-1.5$ $450$ $20-25$ $75-85$ $250-260$ $90-100$ $80-90$ $1-1.5$ $450$ $20-25$ $75-85$ $250-260$ $25-214$ $41-86$ $1-1.5$ $460$ $40-45$ $60$ $37-386$ $55-70$ $26-506$ $80-90$ $1-1.5$ $5600$ $40-45$ $60$ $37-386$ $55-124$ $41-86$ $1-1.5$ $5600$ $40-45$ $50-126$ $25-124$ $30-90$ $29-71$ $1-2$ $38-57$   | *A. fuscosuccinea   | 0.5 - 0.8     | 60-80            | 25-35       | 10-15        | 140 - 150                      | 30-50         | 150 - 160    | 60-70         | 70–80        | 50-60 	imes 4-5 cy  | $12-14 \times 4-5$ a               |
| nea $1-4$ $38-136$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $0.65-0.8$ $30-80$ $8-9$ $40-45$ $300$ $75-80$ $220-230$ $30-35$ $70$ $1-4$ $865-897$ $13-45$ $30-111$ $82-607$ $32-361$ $26-506$ $55-224$ $41-86$ $1-1.5$ $450$ $20-25$ $75-85$ $250-260$ $250-260$ $90-100$ $80-90$ $1-1.5$ $450$ $20-25$ $75-85$ $250-260$ $250-260$ $90-100$ $80-90$ $1-1.5$ $>600$ $40-45$ $60$ $370-380$ $65-70$ $440-445$ $65-70$ $80-90$ $1-3$ $38-132$ $15-50$ $9-45$ $64-135$ $38-57$ $52-124$ $39-98$ $29-71$ $1-2$ $40$ $58-114$ $27-81$ $130$ $70-85$ $99-264$ $24-45$ $56-135$ $0.8-10$ $68-100$ $19-210$ $19-28$   | †A. fuscosuccinea   | 0.3 - 0.4     | 35-90            | 30-35       | 50-55        | 06                             |               | 100          | 40            | 40           | 55-60 	imes 3-3.5   | $7.5-8.5 \times 4$ cu/e            |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$  | •A. fuscosuccinea   | 1-4           | 38-136           | 13-45       | 30-111       | 82-607                         | 32-361        | 26-506       | 55-224        | 41–86        | $45-59 \times 3-6.5$  | $11-13.6 \times 6.5-8.5 a$         |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | †A. minor           | 0.65 - 0.8    |                  | 8–9         | 40-45        | 300                            | 75-80         | 220-230      | 30-35         | 70           | 70 	imes 5  | $7-8 \times 3-4$                   |
| $ \begin{array}{cccccccccccccccccccccccccccccccccccc$   | •A. nigricans       | 1-4           |                  | 13-45       | 30-111       | 82-607                         | 32–361        | 26-506       | 55-224        | 41–86        | 52-73 	imes 3.2-6.5   | $14.5 - 17 \times 5 - 7a$          |
| $\begin{array}{cccccccccccccccccccccccccccccccccccc$  | *A. polytricha      | 1 - 1.5       |                  | 20-25       | 75-85        | 250-260                        | 250           | 250–260      | 90-100        | 80–90        | 50-60 	imes 4-5 cy  | $12-15 \times 5-6$                 |
| 1-3     38-132     15-50     9-45     64-135     38-57     52-124     39-98     29-71       1-2     40     58-114     27-81     130     70-85     99-264     24-45     56-135       0.8-1     85-100     40-50     20-30     195-210     190-210     170-185     20-30     80-90       0.8-1     85-100     40-50     20-30     195-210     190-210     170-185     20-30     80-90       0.34-07     <80   | †A. polytricha      | 1 - 1.5       | >600             | 40-45       | 60           | 370–380                        | 65-70         | 440–445      | 65-70         | 80-85        | $65-90 \times 4.5-6 \text{ cy}$   | $12.5 - 15.5 \times 6.5 - 7$ f/e   |
| 1-2         40         58-114         27-81         130         70-85         99-264         24-45         56-135           0.8-1         85-100         40-50         20-30         195-210         190-210         170-185         20-30         80-90           0.34-07         <80  | •A. scissa          | 1–3           | 38-132           | 15-50       | 9-45         | 64-135                         | 38-57         | 52-124       | 39–98         | 29–71        | $30-44 \times 3.7-4.9$ cl   | $8.7 - 13 \times 3.7 - 5.7$ a      |
| 0.8-1 85-100 40-50 20-30 195-210 190-210 170-185 20-30 80-90<br>0.34-07 <80 23-46 10-29 35-181 31-98 98-307 21-78 44-78   | •A. subglabra       | 1 - 2         | 40               | 58-114      | 27-81        | 130                            | 70-85         | 99–264       | 24-45         | 56-135       | $33-41 \times 3.6-5.1$ cl   | $10-11.5 \times 4.5-5.5$ a         |
| 0.34-0.7 < 80 $3.3-46$ $10-29$ $3.5-181$ $31-98$ $98-307$ $21-78$ $44-78$   | *†A. tenius         | 0.8 - 1       | 85-100           | 40–50       | 20–30        | 195-210                        | 190-210       | 170-185      | 20–30         | 80–90        | 50-60 	imes 4-6   | $12-15 \times 5-6$ e/o             |
|   | A. thailandica      | 0.34-0.7      | ≤80              | 23-46       | 10–29        | 35-181                         | 31–98         | 98–307       | 21–78         | 44–78        | $34.67 - 48.41 \times 2.73 - 6.31$ cy $9.47 - 13.10 \times 4.45 - 5.66$ a   | $9.47{-}13.10 \times 4.45{-}5.66a$ |

TABLE 2. Synopsis of dimensions of basidiocarps, internal zones, basidia and spores of A. thailandica and similar species of Auricularia. TH=thickness of basidiocarp, ZP=zona pilosa, ZC=zona

Though the characters of the hairs are certainly not a sufficient criterion to allow a diagnosis of a species (Lowy, 1951), hairs of *A. thailandica* show considerable differences from other species with a medulla. Though the size of the zona pilosa of *A. thailandica* is similar to *A. fibrillifera*, *A. fuscosuccinea*, *A. minor*, *A. scissa* and *A. subglabra* (Table 2), the loosely (scattered) arranged abhymenial hairs of *A. thailandica* are a useful character to distinguished this latter species. The nature of hair tips, size of hair walls and size of basidiocarp are also supportive characters which can distinguish *A. thailandica* from similar species. In the ITS phylogeny all the specimens from different geographic locations group in a monophyletic clade and *A. thailandica* is a distinct species with high bootstrap support. In the present study we used both morphological and molecular data to define *A. thailandica* as a distinct species (Looney *et al.*, 2013; Malysheva & Bulakh, 2014; Wu *et al.*, 2014b).

The ITS region can be used to define species of *Auricularia* corresponding to their morphology (Looney *et al.*, 2013; Weiß & Oberwinkler, 2001) and can also resolve interspecies relationships. Combining the gene analyses of ITS and rpb2 (Looney *et al.*, 2013) and ITS and LSU has been used in phylogenetic analyses in different studies (Weiß & Oberwinkler, 2001). However, considering both morphological and phylogenetic analysis, ITS appears to be an adequate molecular marker for phylogenetic species delimitation in *Auricularia*.

*Auricularia* is the fourth most cultivated mushroom genus after *Agaricus, Lentinula* and *Pleurotus* (Chang, 1996). Due to its nutritional value, medicinal value, and general appreciation as a table delicacy, the importance of this mushroom has increased rapidly (Miles & Chang, 2004). The fruiting bodies of *Auricularia* are a rich source of polysaccharides (De Silva *et al.*, 2012a; Miles & Chang, 2004) and possess antioxidant activities, anticoagulant activities and decrease blood sugar levels (De Silva *et al.*, 2012b; De Silva *et al.*, 2013; Mortimer *et al.*, 2014). The new species, *A. thailandica* may have the potential for domestication as a new edible mushroom that grows in tropical climates (Thawthong *et al.*, 2014).

Currently, 163 names of *Auricularia* have been recorded in Index Fungorum (2015), but many are synonyms, invalid names or species variants. Out of 163 records 75 are possibly valid names (Wu *et al.*, 2014a). Due to a lack of taxonomic and phylogenetic investigations, the number of species of *Auricularia* is still poorly known, particularly from the tropics; therefore future research should focus on investigating the genus *Auricularia* in tropical and subtropical areas.

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# References

- Chandrasrikul, A., Suwanarit, P., Sangwanit, U., Morinaga, T., Nishizawa, Y. & Murakami, Y. (2008) *Diversity of mushrooms and macrofungi in Thailand*. Kasetsart University Press, Thailand, 514 pp.
- Chang, S.T. (1996) Mushroom research and development-equality and mutual benefit. *Mushroom Biology and Mushroom Products*: 1–10.
- Daep, N. & Cajuday, L. (2003) Mushroom Diversity at Mt Malinao, Albay. PSSN Nature News 2: 57.
- De Leon, A.M., Luangsa-ard, J.J.D., Karunarathna, S.C., Hyde, K.D. & Reyes, R.G. (2013) Species listing, distribution, and molecular identification of macrofungi in six Aeta tribal communities in Central Luzon, Philippines. *Mycosphere* 4 (3): 478–494.
- De Silva, D.D., Rapior, S., Fons, F., Bahkali, A.H. & Hyde, K.D. (2012a) Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action. *Fungal Diversity* 55 (1): 1–35. http://dx.doi.org/10.1007/s13225-012-0151-3

De Silva, D.D., Rapior, S., Hyde, K.D. & Bahkali, A.H. (2012b) Medicinal mushrooms in prevention and control of diabetes mellitus.

Fungal Diversity 56 (1): 1–29.

http://dx.doi.org/10.1007/s13225-012-0187-4

- De Silva, D.D., Rapior, S., Sudarman, E., Stadler, M., Xu, J., Alias, S.A. & Hyde, K.D. (2013) Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. *Fungal Diversity* 62 (1): 1–40. http://dx.doi.org/10.1007/s13225-013-0265-2
- Duncan, E. & MacDonald, J. (1967) Micro-evolution in Auricularia auricula. Mycologia 59 (5): 803–818. http://dx.doi.org/10.2307/3757193
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Jones, E.B.G., Whalley, A.J.S. & Hywel-Jones, N.L. (1994) A fungus foray to Chiang Mai market in northern Thailand. *Mycologist* 8 (2): 87–90.

http://dx.doi.org/10.1016/S0269-915X(09)80139-0

- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30 (4): 772–780. http://dx.doi.org/10.1093/molbev/mst010
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (Eds.) (2008) Anisworth & Bisby's Dictionary of the Fungi. CAB International, Oxon, UK, pp. 68–69.
- Klomklung, N., Karunarathna, S., Chukeatirote, E. & Hyde, K. (2012) Domestication of wild strain of Pleurotus giganteus. *Sydowia* 64 (1): 39–53.
- Kobayashi, Y. (1981) The genus Auricularia. Bulletin of the National Science Museum, Tokyo 7 (2): 41-67.
- Kornerup, A. & Wanscher, J.H. (1978) Methuen Handbook of Colour. Eyre Methuen, London, UK, 252 pp.
- Lalap, A. (1981) Species of Auricularia in the Philippines. Master of Science Thesis, University of the Philippines Los Baños.
- Looney, B.P., Birkebak, J.M. & Matheny, P.B. (2013) Systematics of the genus *Auricularia* with an emphasis on species from the southeastern United States. *North American Fungi* 8: 1–25. http://dx.doi.org/10.2509/naf2013.008.006
- Lowy, B. (1951) A morphological basis for classifying the species of *Auricularia*. *Mycologia* 43 (3): 351–358. http://dx.doi.org/10.2307/3755598
- Lowy, B. (1952) The genus Auricularia. Mycologia 44 (5): 656-692.
- Malysheva, V.F. & Bulakh, E.M. (2014) Contribution to the study of the genus *Auricularia (Auriculariales, Basidiomycota)* in Russia. *Novosti Sistematiki Nizshikh Rastenii* 48: 164–180.
- Miles, P.G. & Chang, S.T. (2004) *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact.* CRC press, Boca Raton, Florida, 480 pp.
- Montoya-Alvarez, A.F., Hayakawa, H., Minamya, Y., Fukuda, T., López-Quintero, C.A. & Franco-Molano, A.E. (2011) Phylogenetic relationships and review of the species of *Auricularia* (Fungi: Basidiomycetes) in Colombia. *Caldasia* 33 (3): 55–66.
- Mortimer, P.E., Xu, J., Karunarathna, S.C. & Hyde, K.D. (Eds.) (2014) *Mushrooms for trees and people: a field guide to useful mushrooms of the Mekong region*, The World Agroforestry Centre, East Asia, Kunming, China, pp. 125.
- Musngi, R.B., Abella, E.A., Lalap, A.L. & Reyes, R.G. (2005) Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *Journal of Agricultural Technology* 1 (2): 279–299.
- Nylander, J.A.A. (2004) MrModeltest version 2. Program distributed by the author. *Evolutionary Biology Centre, Uppsala University* 2: 1–2.
- Parmasto, E. & Parmasto, I. (1987) Variation of basidiospores in the hymenomycetes and its significance to their taxonomy. *Bibliotheca Mycologica* 115: 1–168.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes version 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–542.

http://dx.doi.org/10.1093/sysbio/sys029

- Ruksawong, P. & Flegel, T.W. (2001) *Thai mushrooms and other fungi*. National Science and Technology Development Agency, Bangkok, Thailand, 268 pp.
- Sibounnavong, P., Cynthia, C., Kalaw, S., Reyes, R. & Soytong, K. (2008) Some species of macrofungi at Puncan, Carranglan, Nueva Ecija in the Philippines. *Journal of Agricultural Technology* 4 (2): 105–115.
- Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12 (4): 335–337. http://dx.doi.org/10.1007/s13127-011-0056-0
- Swofford, D.L. (2003) *PAUP\**. *Phylogenetic analysis using parsimony (\*and other methods)*. version 4. Sinauer Associates, Sunderland, Massachusetts.

- Thawthong, A., Karunarathna, S.C., Thongklang, N., Chukeatirote, E., Kakumyan, P., Chamyuang, S., Rizal, L.M., Mortimer, P.E., Xu, J., Callac, P. & Hyde, K.D. (2014) Discovering and domesticating wild tropical cultivatable mushrooms. *Chiang Mai Journal of Science* 41 (4): 731–764.
- Wang, X., Yao, F., Zhang, Y., Fang, M. & Chen, Y. (2013) Feasibility Research on ITS Sequence as DNA Barcode of *Auricularia*. Journal of Northeast Forestry University 41 (7): 111–114.
- Weiß, M. & Oberwinkler, F. (2001) Phylogenetic relationships in Auriculariales and related groups-hypotheses derived from nuclear ribosomal DNA sequences. *Mycological Research* 105 (4): 403–415. http://dx.doi.org/10.1017/S095375620100363X
- Wen, Y.L., Cao, H. & Pan, Y.J. (2005) Application of ERIC method to the affinity among *Auricularia* strains. *Mycosystema* 24 (1): 53–60.
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, California, pp. 315–322. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1
- Wong, G. & Wells, K. (1987) Comparative morphology, compatibility, and interfertility of *Auricularia cornea*, A. polytricha, and A. tenuis. Mycologia 79 (6): 847–856. http://dx.doi.org/10.2307/3807686
- Wu, F., Yuan, Y., Liu, H.G. & Dai, Y.C. (2014a) Auricularia (Auriculariales, Basidiomycota): a review of recent research progress. Mycosystema 33 (2): 198–207.
- Wu, F., Yuan, Y., Malysheva, V.F., Du, P. & Dai, Y.C. (2014b) Species clarification of the most important and cultivated *Auricularia* mushroom "Heimuer": evidence from morphological and molecular data. *Phytotaxa* 186 (5): 241–253. http://dx.doi.org/10.11646/phytotaxa.186.5.1
- Yan, P.S., Jiang, J.H., Wang, D.C., Luo, X.C. & Zhou, Q. (2002) Molecular taxonomic relationship of *Auricularia* species inferred from RAPD markers. *Mycosystema* 21 (1): 47–52.
- Yan, P.S., Luo, X.C. & Zhou, Q. (1999) RFLP analysis of amplified nucleur ribosomal DNA in the genus Auricularia. Mycosystema 18 (2): 206–213.
- Yan, P.S., Luo, X.C. & Zhou, Q. (2004) RAPD molecular differentiation of the cultivated strains of the jelly mushrooms, *Auricularia auricula* and *A. polytricha. World Journal of Microbiology and Biotechnology* 20 (8): 795–799. http://dx.doi.org/10.1007/s11274-004-5840-y