**Phallus chiangmaiensis** sp. nov. and a Record of *P. merulinus* in Thailand

Sujinda Sommai, Phongsawat Khamsuntorn, Sayanh Somrithipol, Janet Jennifer Luangsa-ard and Umpawa Pinruan

Plant-Microbe Interaction Research Team (APMT), Integrative Crop Biotechnology and Management Research Group (ACBG), BIOTEC, National Science and Technology Development Agency, Pathum Thani, Thailand

**ABSTRACT**

During the rainy season in Thailand, specimens of *Phallus chiangmaiensis* sp. nov. and *P. merulinus* were collected from Chiang Mai and Samut Sakhon Provinces, respectively. Molecular phylogenetic analyses based on sequences of the nuclear ribosomal large subunit (LSU), nuclear ribosomal 5.8S gene including the internal transcribed spacer regions 1 and 2 (ITS), and the protein-coding gene *atp6* (mitochondrial adenosine triphosphate [ATP] synthase subunit 6) support the placement of the new species within *Phallus*. *Phallus chiangmaiensis* has a well-developed white indusium and campanulated caps with reticulate surfaces. It differs morphologically from the related species, as supported by the phylogenetic data. *Phallus merulinus* is reported here as a species that was re-encountered in Thailand. The descriptions of the species are accompanied by illustrations of macro- and micro-morphological features, and a discussion of the related taxa is presented.

**INTRODUCTION**

Species in the genus *Phallus* Junius ex L., commonly known as stinkhorn, are gasteroid fungi in the family Phallaceae, order Phallales, with *P. impudicus* L. as the type species. The genus is characterized by a fetid odor originating from the gleba. The important morphological features used for species delimitation are the shape and surface configuration of the receptacle, the coloration of the receptacle, volva with rhizomorphs, the presence of an erect to curved sponge-like and hollow pseudostipe, the size of the basidiomata, and the presence or absence of indusia (skirt-like structures) [1–3].

Some species of *Phallus*, including *P. atrovolvatus* Kreisel & Calonge, *P. dongsun* T.H. Li, T. Li, Chun Y. et al., *P. echinovolvatus* (M. Zang & Z.X. Hu) Kreisel, *P. fragrans* M. Zang, *P. fuscoechinovolvatus* T.H. Li, B. Song & T. Li, *P. impudicus*, *P. indusiatus* Ventenat., *P. luteus* (Liou & L. Hwang) T. Kasuya, *P. merulinus* (Berk.) Cooke, *P. mengsongensis* H.L. Li, L. Ye, P.E. Mortimer et al., *P. nanchangensis* Z.Z. He, and *P. rubrovolvatus* (M. Zang, D.G. Ji & X.X. Liu) Kreisel are used for food [4–8]. *Phallus rubricundus* (Bosc) Fr. and *P. tenuis* (E. Fisch.) Kuntze are inedible species of *Phallus* that are used as medicines [6,9].

Currently, *Phallus* consists of 95 species, excluding formae, varieties and synonyms, according to the Index Fungorum database (www.indexfungorum.org). *Phallus* is widely distributed in different geographical locations and climate types, such as grasslands, conifer forests, bamboo forests, and broadleaved forests from tropical, subtropical, and temperate areas [3,7,10–17].

During surveys of wild mushrooms in Thailand, we found a new species of *Phallus*, as supported by morphological and phylogenetic analyses. We introduced this new species to the Phallaceae (Phallales, Agaricomycetes). Another species was identified as *Phallus merulinus* which has previously been reported from Thailand [18,19].

**MATERIALS AND METHODS**

**Fungal specimen**

Two fresh specimens from the Saluangnok community forest, Chiang Mai Province, and twelve fresh specimens from Amphoe Ban Phaeo, Samut Sakhon Province, Thailand were collected during the rainy season of 2019.
Isolation and morphological studies

Photographs of the fresh specimens in their natural habitat were taken from different angles with a digital camera (Canon, EOS 60 D, Canon Marketing Co., Ltd., Bangkok, Thailand) for further studies, and field notes relating to possible host plants and the situations in which the fruit bodies were found were documented. The fresh basidiocarps were wrapped in wax paper and carefully handled to a laboratory for isolation. The macroscopic features used for identification, such as color, size, shape, outer surface of the fruiting body, and ecological and host substrates, were recorded. The colors of the fresh specimens were described using The RHS color chart, a sixth revised edition [20].

The small pieces of endoperidium tissue of the fruiting bodies were aseptically transferred to the potato dextrose agar plates (PDA; Difco, Becton, Dickinson and Company, Bangkok, Thailand) with antibiotics (penicillin G (0.05 g/L) and streptomycin sulfate (0.05 g/L)). The plates were incubated at room temperature (25°C). The mycelia emerging from the tissue were transferred to the new PDA plates. The specimens were dried by a dehydration machine at 45°C for 24–36 h and deposited in the BIOTEC Bangkok Herbarium (BBH), Thailand.

The hand section of the dried specimens was made under an Olympus SZ61 (Olympus Co., Ltd., Bangkok, Thailand) and the sections were mounted in 5% KOH solution and 1% Congo Red. Morphological characteristics, such as size, color, and shape of basidiospores; and the cells or hyphae of the cap, pseudostipe, indusium, volva, and rhizomorph, were examined under an Olympus BX31 light microscope. Micrographs were obtained with an Olympus microscope equipped with differential interference contrast (Olympus DP70, Olympus Co., Ltd., Bangkok, Thailand) and a Canon EOS 60 D camera. The growth rate and colony characteristics of the cultures were deposited in the BIOTEC Culture Collection (BCC), Thailand. The fungal taxonomic details were also submitted to Faces of Fungi and Index Fungorum.

DNA extraction and PCR amplification

Genomic DNA was extracted from the mycelia on PDA using a CTAB method [21]. The LSU, ITS, and atp6 gene regions were amplified using the primer pairs LROR/LR5, ITS5/ITS4, and 1M40F/2M, respectively [22–24]. The amplification reactions were performed in a 50 μl reaction volume containing 38.3 μl of ddH2O, 5.0 μl of 10× buffer, 2.5 μl of MgCl2, 1.0 μl of dNTP, 1 μl of each primer (10 μM), 0.2 μl of Taq DNA polymerase (Vivantis, Bang Trading 1992 Co., Ltd., Bangkok, Thailand) and 1 μl of DNA template. The amplification conditions for the LSU and ITS regions followed the protocol described by Sakayaroj [23], while the amplification conditions for the atp6 gene followed the protocol described by Raspé et al. [24]. The PCR products were sequenced using the same primers as used for amplification.

Sequence alignment and phylogenetic analyses

Individual analyses were run for separate loci (ITS dataset consisting of 42 sequences, LSU 34 sequences, atp6 19 sequences) and a combined analysis comprising ITS, LSU, and atp6 (46 sequences) shown in Table 1. Sequences were assembled using BioEdit v.7.0.5.3 [25]. All sequences were aligned with MUSCLE [26] and manually edited using BioEdit v.7.0.5.3 [25]. The phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI).

The maximum likelihood analysis was performed on the CIPRES supercomputer using the program RAxML-HPC2 v.8.2.12 on XSEDE [27]. One thousand nonparametric bootstrap iterations were run with the GTR model and a discrete gamma distribution.

The maximum parsimony analysis was performed by PAUP v.4.0b10 [28] with 10 replicates of stepwise additions, the heuristic search option, 1,000 random taxa addition and the tree-bisection reconnection (TBR) branch-swapping algorithm. All characters were given equal weight, and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed, and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious tree was estimated based on 1,000 bootstrap replications.

The Bayesian analysis was performed MrBayes v.3.0b4 [29] using a uniform [GTR + I + G] model, Isetnst = 6 rates = invgamma; prsetstate-freqpr = dirichlet (1,1,1,1). Four Markov chains were run for 5,000,000 generations, and trees were sampled every 100 generations. The first 5,000 trees, which represented the burn-in phase of the analysis, were discarded, with 50,000 trees used for calculating posterior probabilities (BIPP) in the consensus tree.

Results

Phylogenetic analyses

The ITS dataset included 42 sequences, and Mutinus albotruncatus (UFRN Fungos 2025) was used as an outgroup [30]. The best scoring of the RAxML tree
Phallus chiangmaiensis sp. nov. (BCC 92054), are closely related to *P. echinovolvatus* with bootstrap and posterior probability strong support (94% BSML, 98% BSMP and 0.99 BPP), shown in Figure 1. However, the morphological analyses that our new species and *P. echinovolvatus* are distinct. Phylogenetic trees generated from LSU and atp6 sequences can be seen in Supplementary Figures 1 and 2.

The dataset of combined genes (ITS, LSU, and atp6) of *M. albotruncatus* (UFRN Fungos 2025) was used as an outgroup. The best scoring of the RAxML tree is shown in Figure 2, with the final optimization likelihood value of $-11436.786086$. The maximum parsimony dataset consists of 2,438 characters, of which 1,737 steps were informative with a length of 1,715 steps (CI = 0.375). Bootstrap support values for maximum likelihood (BSML, left), maximum parsimony (BSMP, middle) were >60%. Branches with Bayesian posterior probabilities (BPP, right) >0.95 are indicated at the nodes. The two strains of *Phallus chiangmaiensis* sp. nov. (BCC 92054 and BCC 92055), are closely related to *P. echinovolvatus* with bootstrap and posterior probability strong support (94% BSML, 98% BSMP and 0.99 BPP), shown in Figure 1. However, the morphological analyses that our new species and *P. echinovolvatus* are distinct. Phylogenetic trees generated from LSU and atp6 sequences can be seen in Supplementary Figures 1 and 2.

The dataset of combined genes (ITS, LSU, and atp6) of *M. albotruncatus* (UFRN Fungos 2025) was used as an outgroup. The best scoring of the RAxML tree is shown in Figure 2, with the final optimization likelihood value of $-11436.786086$. The maximum parsimony dataset consists of 2,438 characters, of which 1,715 were constant, 208 were variable parsimony-uninformative and 515 were parsimony informative with a length of 1,737 steps (CI = 0.625, RI = 0.770, RC = 0.481 and HI = 0.375). Bootstrap support values for maximum likelihood (BSML, left), maximum parsimony (BSMP, middle) were >60%. Branches with Bayesian posterior probabilities (BPP, right) >0.95 are indicated at the nodes. The phylogenetic analyses showed that all the collected strains were clustered in the family Phallaceae. The two strains of *Phallus chiangmaiensis*
sp. nov. (BCC 92054 and BCC 92055), which were recovered as a distinct species, grouped with *P. echino-volvatus*, *P. fuscoechinovolvatus*, *P. multicolor*, *P. luteus* and were separated from other species with bootstrap support (99% BSML and 86% BSMP). Both strains of *Phallus merulinus* (BCC 92056 and BCC 92057) clustered with *P. merulinus* (INPA 240010), with high statistical support (100% BSMP, 100% BSML, and 1.00 BPP) in the tree (Figure 2).

**Taxonomy**

*Phallus chiangmaiensis* U. Pinruan, S. Sommai & P. Khamsuntorn, *sp. nov*. Figures 3–5
Index Fungorum number: IF557726; Facesoffungi number: FoF 08402

**Etymology:** The name refers to Chiang Mai Province, the location where the mushroom was collected.

Asexual morph: Unknown.

Holotype: BBH 47825

**Sexual morph:** Egg globose to subglobose, 22–30 mm in diam., white (RHS2015 N155C) with a

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**Figure 2.** Phylogenetic relationships of *Phallus* spp. from a combined ITS, LSU, and atp6 analyses. Numbers at the significant nodes represent ML bootstrap values/MP/Bayesian posterior probabilities, multiplied by 100; bold lines in the tree represent 100% bootstrap (BSMP, BSML) and 1.00 posterior probability (BPP).
white mycelial rhizomorph arising from the base. Exoperidium papery, milky white (RHS2015 N155C); mesoperidium gelatinous or lightly viscous, transparent to subtransparent, 3.5–5 mm thick, moderate yellowish-brown (RHS2015 N199C); endoperidium membranous, thin, white (RHS2015 N155C), covering an upper surface of gleba. Mature basidiomata 205–215 mm high. Cap campanulate, 40–50 mm high, 35–45 mm wide, surface strongly reticulate, light yellow (RHS2015 162C), meshes deep, polygonal, apex with an apical pore and covered with greenish-white (RHS2015 155C) membrane approximately \( \frac{1}{4} \) the size of the cap. Gleba moderate olive-brown (RHS2015 199A), mucilaginous. Pseudostipe 158–165 mm high, cylindrical, tapering toward the apex, 20–25 mm wide at the base, 10–13 mm wide at the apex, white (RHS2015 NN155D), fragile and soft, spongy, hollow. Indusium coarsely latticed, white (RHS2015 NN155D), extended to \( \frac{3}{4} \) the size of the pseudostipe. The meshes of indusium are large, hexagonal or polygonal, 5–10 mm wide. Volva globose to subglobose, 55 × 40 mm in diam., light brownish gray (RHS2015 201B), smooth surface. Rhizomorphs white (RHS2015 NN155D), when scratched the color changes to light purple (RHS2015 85B). Odor fetid.

**Basidia** 7.0–15.0 × 1.5–3.0 μm, elongated, cylindrical, slightly broader at the center, hyaline. Sterigmata 4–8 in number. Basidiospores 3.0–4.0 × 1.5–2.0 μm (\( x = 3.9 \times 1.9 \) μm, \( n = 55 \)), ellipsoid, greenish-white (RHS2015 192D) in 5% KOH, inamyloid, smooth surface and thin-walled. Cap cells and hyphae; cells 12.5–25 μm in diam.,

**Figure 3.** Phallus chiangmaiensis (BBH 47825, holotype). (a) Mature basidiomata. (b) Reticulate cap. (c) Indusium. (d) Immature basidiomata (egg). (e) Pseudostipe and section of immature basidiomata. Scale bars: a = 50 mm, b–e = 10 mm.
globose to subglobose, hyaline, thin-walled; hyphae 2.0–10.0 μm wide, hyaline, thin-walled, septate, branched with clamp connections. Cells of pseudostipe 15.0–67.5 μm in diam., pseudoparenchymatous, globose to a subglobose, bubble-like, hyaline, smooth surface and thin-walled. Cells of indusium 12.5–57.5 μm in diam., hyaline, globose to subglobose or bubble-like, smooth surface, thin-walled. Volva hyphae composed of two types of hyphae; type I: 2.0–2.5 μm wide, hyaline, septate, branched,
smooth surface, thin-walled with clamp connections, type II: 5.0–10.0 μm wide, irregular shape, hyaline, septate, branched smooth surface and thick-walled; crystal deposits in globose to subglobose cells distributed among the hyphae. **Rhizomorph hyphae** are composed of two types of hyphae; type I: 2.5–5.0 μm wide, hyaline, septate, branched, smooth surface, thin-walled with clamp connections, type II: 10.0–15.0 μm wide, hyaline, branched, smooth surface, thin-walled, swollen at the tip.

**Known distribution:** Saluangnok community forest, Amphoe Mae Rim, Chiang Mai Province, Thailand.

Habit and Habitat: Solitary or scattered on soil, under *Bambusa* sp.

**Culture characteristics:** Tissue germinated on PDA within 24 h. Colonies were grown on PDA with scant mycelium, entire margin, reaching 2.0 cm in diam. in 1 month at 25°C, surface and reverse white to cream.

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**Materials examined:** THAILAND, Chiang Mai Province, on soil under *Bambusa* sp., October 8, 2019, U. Pinruan, (holotype BBH 47825, isotype BBH 49056); culture ex-holotype BCC 92054, culture ex-isotype BCC 92055.

**Notes:** Phylogenetically, *Phallus chiangmaiensis* is most closely related to *P. echinovolvatus*. Morphologically, it differs from *P. echinovolvatus* on the surface of volva. In *P. echinovolvatus* the volva is echinulate while in *P. chiangmaiensis* it is smooth. The cap of *P. chiangmaiensis* is larger (40–50 × 25–45 mm) than of *P. echinovolvatus* (25–30 × 25–30 mm). The length of indusium in *P. chiangmaiensis* is longer (130–160 mm) than in *P. echinovolvatus* (70–100 mm). The basidia of *P. echinovolvatus* are 6–8 μm long with 4–6 sterigmata while those of *P. chiangmaiensis* are up to 15 μm long with up to 8 sterigmata. The phylogenetic analyses show that our new species also grouped with *P. fuscoechinovolvatus*, *P. multicolor*, *P. lutescens* and *P. echinovolvatus*. 

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**Figure 5.** Line drawing of *Phallus chiangmaiensis*. (a) Fruiting body. (b) Cells of indusium. (c,d) Cells of pseudostipe. (e) Volva hyphae. (f) Rhizomorph hyphae. (g) Basidia. (h) Basidiospores. Scale bars: a = 50 mm, b–d = 50 μm, e = 10 μm, f = 20 μm, g–h = 5 μm.
Table 2. Synopsis of macro- and micro- characteristics of *Phallus chiangmaiensis*, *P. echinovolvatus*, *P. fuscoechinovolvatus*, *P. lutescens*, *P. luteus*, and *P. multicolor*.

| Species name | *P. chiangmaiensis* | *P. echinovolvatus* | *P. fuscoechinovolvatus* | *P. lutescens* | *P. luteus* | *P. multicolor* |
|--------------|---------------------|---------------------|--------------------------|----------------|-------------|----------------|
| **Cap shape** | Campanulate         | Campanulate         | Ovoid to slightly conical or campanulate | Conical to slightly campanulate | Campanulate |
| **Cap size (H × W, mm)** | 40 – 50 × 25 – 45 | 25 – 30 × 25 – 30 | 22 – 40 × 10 – 22 | 15 – 20 × 16 – 23 | 25 – 40 × 26 – 38 | 25 – 30 × 20 |
| **Cap color** | Light yellow        | Nearly white to yellow | Yellowish white | Pale yellow to yellowish orange | Yellow to yellowish orange | Lemon yellow |
| **Cap surface characters** | Strongly reticulated | Reticulated | Strongly rugose | Reticulated with irregular ridges | Strongly reticulated | Reticulated |
| **Gleba color** | Moderate olive brown | Olive to dark brown | Olivaceous brown | Olive brown | Olivaceous brown to greenish black | Olive brown |
| **Indusium length (mm)** | 130 – 160 or ⅔ the size of the pseudostipe | 70 – 100 | ¾ the size of the pseudostipe | Expanded to ⅔–⅔ portion of pseudostipe | 60 – 160 | 78 |
| **Indusium color** | White               | White              | White          | Yellow to yellowish orange | Lemon yellow to yellowish orange |
| **Indusium characters (meshes)** | Hexagonal or polygonal | Polygonal | Hexagonal or polygonal | Polygonal | N/A |
| **Pseudostipe shape** | Cylindrical, tapering toward the apex | Cylindrical to fusiform | Cylindrical or fusiform | Cylindrical usually tapered upwards and enlarged downwards | N/A |
| **Pseudostipe size (mm)** | 158 – 165 × 20 – 25 | 90 – 150 × 20 – 30 | 80 – 130 × 15 – 20 | Snow white to very weak cream white | 70 – 220 × 15 – 25 | 60 – 85 × 25 – 30 |
| **Pseudostipe color** | White | Nearly white | Snow white to milky white | Snow white to very weak cream white | N/A | Yellowish white |
| **Pseudostipe characters** | Spongy, hollow, elongated, cylindrical, a bit broader at the center, and hyaline. Sterigmata 4–8 in number | Hollow | Hollow | Spongy, hollow | Spongy, hollow |
| **Volva characters** | Non-echinulate | Echinulate | Echinulate | Smooth or lightly rugose | Non-echinulate |
| **Basidia** | N/A | N/A | N/A | N/A | N/A |
| **Spore shape** | Cylindrical to broadly ellipsoidal | Oval to ellipsoidal | Cylindrical to broadly ellipsoidal | Cylindrical to long ellipsoidal | Broadly ellipsoid to cylindrical |
| **Spore size (μm)** | 3.0 – 4.0 × 1.5 – 2.0 | 3.0 – 4.0 × 1.3 – 2.0 | 2.5 – 4.0 × 1.0 – 2.0 | 3.0 – 4.3 × 1.1 – 1.8 | 3.0 – 4.0 × 1.5 – 2.0 | 3.94 – 4.33 × 1.77 – 1.97 |
| **Spore color** | Greenish white | Light brownish green to olive | Hyaline and very light olivaceous | Hyaline and light olivaceous | Hyaline | Hyaline |

MYCOBIOLOGY
However, it morphologically differs from *P. multicolor* and *P. luteus* in having a white indusium, and from *P. fusceochinovolvatus* in having non-echinulated volva, as shown in Table 2.

**Phallus merulinus** (Berk.) Cooke (1882)

Figures 6–8

**Basionym:** Dictyophora merulina Berk. (1886)

**Synonyms:**

≡ Clautriavia merulina (Berk.) Lloyd (1909)

≡ Dictyophora irpicina Pat. (1898)

≡ Phallus irpinus (Pat.) Lloyd (1907)

Notes on morphology from Thai specimens: Egg globose to subglobose, 40–50 mm in diam., dark grayish yellowish brown to light gray (RHS2015 N200A to N200D) with a white mycelial rhizomorph arising from the base. Exoperidium papyraceous, light brownish gray (RHS2015 N200C); mesoperidium gelatinous or lightly viscous, transparent to subtransparent,
3–5 mm thick, dark grayish yellow (RHS2015 N199D); endoperidium membranous, thin, white (RHS2015 N155D), covering the upper surface of gleba. **Mature basidiomata** 120–160 mm high. **Cap** campanulate, incurved toward the pseudostipe, surface very densely and merulioid-wrinkled, sticky, 10–30 mm high, 10–30 mm wide, light yellow or moderate yellow (RHS2015 160B or 161A), apex round to truncate with an apical pore. **Gleba** light olive brown (RHS2015 199B), mucilaginous. **Pseudostipe** 100–160 mm high, cylindrical, tapering, 13–35 mm wide at the base, 10–25 mm wide at the base.

**Figure 7.** Microscopic features of *Phallus merulinus*. (a–e) Cap cells and hyphae. (f) Cells of indusium. (g) Cells of pseudostipe. (h,i) Volva hyphae. (j,k) Rhizomorph hyphae. (l) Basidiospores. (m) Colony on PDA (surface and reverse plate). Scale bars: a–b, f–g = 20 μm, c–e, h–l = 5 μm, m = 20 mm.
apex, white (RHS2015 NN155D), fragile, soft and spongy, hollow. Indusium coarsely latticed, white (RHS2015 NN155D), extended to \( \frac{1}{3} \) the size of the pseudostipe. The meshes of the indusium are large, polyhedral to round, 2–5 mm wide, the upper meshes larger than the lower meshes, the lower mesh margin wavy and thin. Volva subglobose, incurved toward the pseudostipe, 40–50 mm in diam., light brownish gray (RHS2015 200C), scar surface, with mycelial rhizomorphs from the base. Odor fetid.

Basidiospores 3.5–4.5 × 1.2–1.5 \( \mu \)m (\( \bar{x} = 4.2 \times 1.3 \) \( \mu \)m, \( n = 25 \)), subcylindrical to long-ellipsoid, subhyaline, smooth, thin-walled. Cap cells and hyphae; cells 10–50 \( \mu \)m in diam., globose to subglobose, hyaline, thin-walled; hyphae 2–3 \( \mu \)m wide, hyaline, thin-walled, septate, branched with clamp connections. Cell of pseudostipe 22–63 \( \mu \)m in diam., globose to subglobose, hyaline, smooth surface, thin-walled, bubble-like. Cell of indusium 20–57 \( \mu \)m in diam., globose to subglobose, hyaline, smooth surface, thin-walled, bubble-like. Volva hyphae; outer layer 3.75–10.0 \( \mu \)m wide, pale brown to brown, branched, smooth surface, thin-walled, septate with clamp connections; inner layer 1.2–7.5(20) \( \mu \)m wide, hyaline, branched, smooth surface, thin-walled, septate with clamp connections, swollen at the tip. Rhizomorph hyphae; outer layer 2.5–7.5 \( \mu \)m wide, hyaline to a pale brown, septate, branched, smooth surface, thick-walled with clamp connections; inner layer 1.2–7.5 \( \mu \)m wide, hyaline, branched, septate, smooth surface, thin-walled with clamp connections.

Known distribution: Australia [31], Brazil [32], China [33], French Guiana [34], India [35,36], Indonesia [37–42], Philippines [43], Republic of Trinidad and Tobago [18], Sri Lanka [44–48], Thailand [18].

Habit and Habitat: on decomposing rice straw. Culture characteristics: Tissue germinated on PDA within 24 h. Colonies were grown on PDA, immersed mycelium, reaching 2 cm in diam. in 1 month at 25°C, surface and reverse white to cream.

Figure 8. Line drawing of Phallus merulinus. (a) Fruiting body. (b,c) Cap cells and hyphae. (d) Cells of indusium. (e,f) Cells of pseudostipe. (g) Volva hyphae. (h) Rhizomorph hyphae. (i) Basidiospores. Scale bars: a = 20 mm, b, g = 10 \( \mu \)m, c, h, i = 5 \( \mu \)m, d = 20 \( \mu \)m, e–f = 50 \( \mu \)m.
Materials examined: THAILAND, Samut Sakhon Province, on decomposing rice straw, September 7 2019, U. Pinruan, (BBH 47826, BCC 92056; BBH 49055, BCC 92057; BBH 49057; BBH 49058, BBH 49059, BBH 49060, BBH 49061, BBH 49062, BBH 49063, BBH 49064, BBH 49065, BBH 49066).

Notes: The combined sequences of Phallus merulinus (BCC 92056 and BCC 92057) are identical to those of P. merulinus (INPA 240010), with 100% BSMP, 100% BSML, and 1.00 BPP. Phallus merulinus is the most distinctive among the Phallus species, mainly due to its cap, which has merulioïd-wrinkled on the surface, and pale volva whereas most Phallus species have conspicuously reticulate indusium and dark volva.

Discussion

At present, most taxonomic studies of Phallus have been based on morphological features and molecular analyses. In this study, we introduce a new species, Phallus chiangmaiensis, based on its unique macro- and micro- morphological characteristics together with the support of molecular phylogenetic analyses. This species is morphologically related to the well-known species, P. indusiatus (Vent.) Desv. They have a campanulate cap with a reticulated surface and a pore at the apex. Their gleba are mucilaginous and moderately olive-brown. They have a white pseudostipe and a well-developed net-like, white indusium without a serrated margin. Their volva are non-echinulate and have rhizomorphs. However, spores of the new species are greenish-white, while those of P. indusiatus are hyaline. Phallus indusiatus has smaller basidiocarps and capsizes than those of the new species (basidiocarps: 15–20 mm; cap sizes: 18–32 × 16–27 mm). The pseudostipe and indusium length of P. indusiatus are shorter than those of the new species (pseudostipe: 75–110 × 11–22 mm; indusium: 100–200 mm) [17]. Moreover, the new species has caps covered with a greenish white membrane and hyaline basidia, which are not observed in P. indusiatus. Apart from the morphology, the molecular phylogenetic analysis revealed that the two species were separate.

In the molecular phylogenetic analyses, which were based on sequences of the ITS, LSU, and atp6 gene regions, this species was well separated from other Phallus species with high bootstrap support values (Figures 1 and 2). The most closely related species in the phylogenetic trees are P. echinovolvatus, P. fuscoechinovolvatus, P. multicolor (Berk. & Broome) Cooke, and P. luteus. Phallus echinovolvatus and P. fuscoechinovolvatus are similar to the new species in having a long white indusium, but they differ in having echinulate volva [11,17,49,50]. Phallus chiangmaiensis always has non-echinulate and milk white volva, that is never in blackish or black color. Phallus multicolor has a lemon yellow to yellowish orange indusium and a yellowish white pseudostipe [51,52] while the new species has white indusium and pseudostipe which differs. Phallus luteus has a yellowish orange indusium and a pale pink to reddish purple volva [14] while the new species differs in having white indusium and milk white volva. Their morphological comparison is summarized in Table 2.

The findings of our study appear to be represented a re-encounter of Phallus merulinus 93 years after its first record in Thailand. Both the macroscopic and microscopic features of our specimens agree well with previous descriptions [18,34,36]. The molecular phylogenetic trees revealed that four sequences of P. merulinus were related to P. atrovolvatus. Morphologically, P. merulinus is similar to P. atrovolvatus in having a rugulose to merulioïd cap surface and white indusium. They have closely sizes of spores. However, the volva of P. atrovolvatus appears to be globose to ovoid shapes (19–47 × 18–29 mm) and always blackish color [53,54], while volva shapes of P. merulinus are globose to subglobose, and slightly larger (40–50 mm in diam.), and the volva color always paler [34,52,55]. Phallus merulinus can be also separated from P. atrovolvatus by the unpleasant smell of the gleba (odor fetid) while the gleba odor of the latter species are strong, sweet, and aromatic (but never fetid) [54].

Several species of Phallus are frequently reported in Thailand (some species reported as Dictyophora) [55–60]. However, P. merulinus was not included in those reports which are considered rare species. The first report in Thailand of the species is in 1977 by Reid [18], and this is the second report.

Dictyophora or Phallus were morphologically separated by the presence or absence of indusium. However, recent molecular phylogenetic analyses revealed most Dictyophora species belong to Phallus [16,49,61–63]. In this study, the phylogenetic analyses (Figures 1 and 2) also show that Phallus species with indusium (P. atrovolvatus, P. chiangmaiensis, P. cinnabarinus, P. denigricans, P. echinovolvatus, P. fuscoechinovolvatus, P. haitangensis, P. indusiatus, P. lutescens, P. luteus, P. merulinus, P. multicolor, P. purpurascens, P. rubrovolvatus, P. serratus, P. squamosus, P. ultraduplicatus) and without (P. calo- ngei, P. campanulatus, P. coronatus, P. dongsun, P. flavocostatus, P. hadriani, P. impudicus, P. mengsonggensis, P. ravenelii, P. rugulosus) are mixed together. Whether Dictyophora is a valid genus or conspecific with Phallus can only be verified if the type of the genus (D. phalloidea from Guiana) has been
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Disclosure statement

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ORCID

Janet Jennifer Luangsa-ard  http://orcid.org/0000-0001-6801-2145
Umpawa Pinruan  http://orcid.org/0000-0001-7553-7548

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