



New species of *Phallus* from a subtropical forest in Xishuangbanna, China

HUILI LI^{1,2,3}, PETER E. MORTIMER^{1,3*}, SAMANTHA C. KARUNARATHNA^{2,4}, JIANCHU XU^{1,3} & KEVIN D. HYDE^{2,4}

¹Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, China

²Institute of Excellence in Fungal Research, and School of Science, Mae Fah Luang University, 57100 Chiang Rai, Thailand

³World Agroforestry Centre, East and Central Asia, 132 Lanhei Road, Kunming 650201, China

⁴Mushroom Research Foundation, 128 M.3 Ban Pa Deng T. Pa Pae, A. Mae Taeng, Chiang Mai 50150, Thailand

*Corresponding author: peter@mail.kib.ac.cn

Abstract

Seven collections of *Phallus* species were made in surveys of a subtropical forest in Xishuangbanna in Yunnan Province, China, during the wet season of 2012. Macro and micro characters, together with nrITS sequence data, were used to separate the collections into three species. *Phallus mengsongensis* and *P. serrata* are introduced as species new to science, while a further *Phallus* species is described, but not formally introduced, due to paucity of material. Macro and micro descriptions, colour photographs, line drawings and nrITS phylogenetic data for all three species are provided, which are discussed in relation to similar species in these genera.

Key words: nrITS, Phallaceae, phylogeny, taxonomy

Introduction

Phallus (= *Dictyophora*) is a major genus within the order *Phallales*. The main characteristics of Phallaceae are development from an 'egg', unbranched, upright fruiting bodies and a foul smelling gleba (Arora 1986). The taxonomic relationship of *Phallus* and *Dictyophora* is confused. The presence of an indusium (or less technically a "skirt" that hangs down from the conical to bell-shaped cap) is a key taxonomic character distinguishing *Dictyophora* and *Phallus* (Cunningham 1944; Arora 1986). Cunningham (1944) and Arora (1986) treat *Phallus* and *Dictyophora* as two independent genera. However, Kreisel (1996) considered *Dictyophora* to be a subgenus of *Phallus sensu lato*. Kreisel (1996) considered that the presence of a pore at the cap (head) and indusium is not sufficient to distinguish the two genera. Recent molecular studies also indicate that the indusium cannot be used as a phylogenetically informative character for the generic level taxonomy of *Phallaceae* (Cabral *et al.* 2012).

Kirk *et al.* (2008) estimated that there are 18 species of *Phallus* worldwide, while Index Fungorum (2013) lists 29 species epithets for *Dictyophora*. Twenty-two species of Phallaceae, belonging to four genera, have been reported from China, 16 of which have been recorded from the Southwest of China (Li *et al.* 2004). Liu *et al.* 2005 listed 13 species of *Phallus* including four varieties of *Phallus costatus* (Penz.) Lloyd (1909: 10) and eight species of *Dictyophora* from China. Species of *Dictyophora* and *Phallus* have a long history of culinary and medical uses, because they are rich in nutrients and bioactive compounds (Lin *et al.* 2011). For example, *Dictyophora rubrovalvata* M. Zang, D.G. Ji & X.X. Liu (1976: 11), known in China as the flower of fungi (Huang 1993), is extremely valuable for its medicinal properties. *Phallus impudicus* L. (1753: 1178) is a prized edible mushroom said to stimulate circulation and have antitumor properties (Lu & Luo 2010). Six *Dictyophora* species and three *Phallus* species are included in the checklist of edible fungi in China (Dai *et al.* 2010).

Although the species of *Phallus* and *Dictyophora* have been used for a long time, scientific investigations regarding their diversity, taxonomy, and phylogeny are rare. The present study uses both molecular and

Materials and Methods

Collecting site and collections

In September and October 2012, seven taxa belonging to *Phallales* (Table 1) were collected from a broad-leaved forest (UTM-N2379744.485281, UTM-E653217.753714) and a tea plantation (UTM-N2378744.485281, UTM-E656217.753714) in Mengsong Village, Xishuangbanna, southern Yunnan, China. The climate is tropical monsoon-influenced, with a dry season and wet season (May to October). In the collection period, these sites were visited twice a week, fresh specimens were photographed in the field, including the substrate on which they were found, and brought to the laboratory for macro-morphological study. Collections were dried using an electric fruit dryer at 40°C for 15h and stored in sealed plastic bags, which were labelled with collection details. The samples are deposited in the Herbarium of the Kunming Institute of Botany (HKAS), Chinese Academy of Sciences, China.

TABLE 1. List of sequences included in the phylogenetic analysis.

Species	GB accession no.	Source	Sequence data	Sequence length
<i>Phallus</i> sp. 1	KF052621	this study	ITS	515bp
<i>Phallus serrata</i>	KF052622	this study	ITS	544bp
<i>P. serrata</i>	KF052623	this study	ITS	502bp
<i>P. mengsongensis</i>	KF052624	this study	ITS	642bp
<i>P. mengsongensis</i>	KF052625	this study	ITS	657bp
<i>P. mengsongensis</i>	KF052626	this study	ITS	627bp
<i>P. mengsongensis</i>	KF052627	this study	ITS	652bp
<i>Mutinus caninus</i>	GQ981513	GenBank	ITS	661bp
<i>P. rugulosus</i>	AF324169	GenBank	ITS	547bp
<i>P. hadriani</i>	DQ404385	GenBank	ITS	501bp
<i>P. phalloidea</i> (<i>Dictyophora phalloidea</i>)	AF324162	GenBank	ITS	568bp
<i>P. impudicus</i>	AF324171	GenBank	ITS	536bp
<i>P. luteus</i> (<i>Dictyophora indusiata</i> f. <i>lutea</i>)	AF324173	GenBank	ITS	546bp
<i>P. echinovolvatus</i> (<i>Dictyophora echinovolvata</i>)	AF324168	GenBank	ITS	567bp
<i>Lysurus cruciatus</i>	AJ878736	GenBank	ITS	642bp
<i>Clathrus ruber</i>	GQ981501	GenBank	ITS	608bp
<i>Ramaria stricta</i>	EU819419	GenBank	ITS	639bp

Morphological study

Macromorphological study. Macro-characters, including the size, shape, colour, surface ornamentation and habitat of fruit body (cap, stipe and volva), were recorded from fresh specimens. Colour terms are described using the Methuen Handbook of Colour (Kornerup & Wanscher 1978).

Micromorphological study. Dried specimens were studied for micromorphological characters using a compound light microscope (Nikon Model Eclipse Ci-s). Hand sections were made under a dissecting microscope (Shanghai Instrument Manufacturer), the mounted in 3%–5% KOH for examination (Yang & Ge 2008). All micromorphological observations and light microscope photographs were carried out at 200×, 400×, and 1000 × using a Nikon compound microscope (Nikon Model Eclipse Ci-s). Spore size, spore colour and the texture of cap, stipe, indusium and volva were recorded and drawings were made. The size ranges of basidiospores viewed in side view are based on at least 25 basidiospore measurements. The drawings were edited in Adobe Photoshop CS3.

Molecular study

DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China) was used to extract the total deoxyribonucleic acid (DNA) from dried herbarium specimens using the instructions supplied. The concentration of DNA was determined using an ultraviolet spectrophotometer.

PCR amplification and sequencing. The PCR amplification was carried out with the primers ITS1–F and ITS4 to amplify the complete Internal Transcribed Spacer (ITS) region (Gardes *et al.* 1993). 20 µl of the reaction mixture contained 2Mix 10 µl, ITS1-F 0.35µl, ITS4 0.35 µl, 50 ng/µl DNA 0.6 µl, ddH₂O 8.7 µl for each sample. The PCR programme was set according to Douanla–Meli *et al.* (2005) with the following modifications: an initial denaturation at 94°C for 3 min, followed by 35 cycles consisting of denaturation at 94°C for 40s, annealing at 55°C for 45s, and extension at 72°C for 1 min, and a final elongation step of 7 min at 72°C. To check the PCR products, 1% agarose gel electrophoresis (AGE) for 30min at 220V was used. All PCR products were sent to Shanghai Majorbio Bio–Pharm Technology Co., Ltd. for purification and sequencing.

Sequence alignment and phylogenetic analysis

The ITS sequences of the seven samples of the genus *Phallus*, and the closely related sequences from GenBank (Table 1), were assembled by SeqMan (DNA Star) and Bioedit followed by manual adjustment to improve alignments. The ITS alignment was done in Clustal X and the phylogenetic analysis was carried out using PAUP 4.0 software. The maximum parsimony trees with bootstrap values were obtained by setting the parameters of the program. The best tree was viewed in Treeview and edited in Adobe Illustrator CS3.

Results and Discussion

Phylogenetic analysis

The analysis of the ITS dataset is presented as a maximum parsimony tree and includes 17 taxa from the genera *Phallus*, *Mutinus*, *Clathrus* and *Lysurus* as the ingroup, and 7 out of 17 were sequenced from our samples while 10 were obtained from the GenBank (Table 1). All the available ITS sequences of *Phallus/Dictyophora* in GenBank were used to build up the phylogenetic tree. *Ramaria stricta* (Pers.) Quél. 1888 was chosen as the outgroup (Fig. 1). The tree reveals four clades: Clade I with 97% bootstrap support comprises four *Phallus* species, Clade II with 100% bootstrap support comprises four *Phallus* collections belonging to one species, Clade III with 94% bootstrap support comprises three *Phallus* species and Clade IV with 99% bootstrap support comprises *Lysurus*, *Clathrus* and *Mutinus*.

The equally weighted maximum parsimony tree, by contrast, showed a consistency index (CI) of 0.680, a retention index (RI) of 0.663, a rescaled consistency index (RC) of 0.451, and a homoplasy index (HI) of 0.320. There are 674 characters in the present analysis, of which 261 are constant, 115 are parsimony-uninformative and 298 (44.2%) are parsimony-informative. In the maximum parsimony tree, the seven collections from Xishuangbanna, grouped into Clade I, KF052622 and KF052623, Clade II, KF052624, KF052625, KF052626 and KF052627, and Clade III, KF052621 (*Phallus* sp. 1) with *Phallus luteus* all with 100% bootstrap support (Fig.1).

Taxonomy

Morphological and phylogenetic analyses indicate that the seven samples from Mengsong, Yunnan Province, belong to *Phallus* sp. 1 (KF052621), *P. serrata* sp. nov (KF052622 and KF052623) and *P. mengsongensis* sp. nov. (KF052624, KF052625, KF052626, and KF052627). *Phallus serrata* and *P. mengsongensis* are proposed as new species to science, while *Phallus* sp. 1 is illustrated and fully described, but not formally introduced due to paucity of the material.

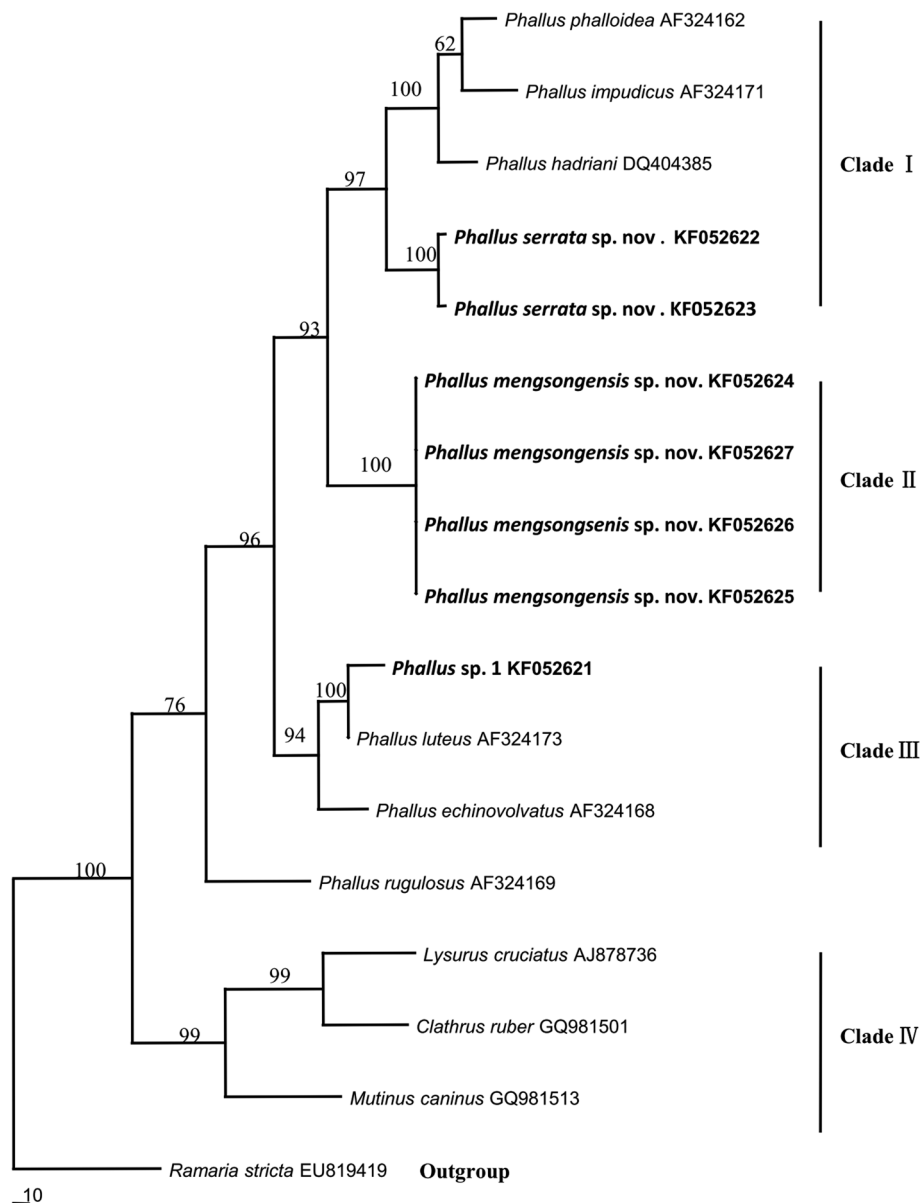


FIGURE 1. Maximum parsimony phylogram based on ITS2 rDNA sequence data showing the phylogenetic position of *P. mengsongensis*, *P. serrata* and *Phallus* sp. 1 with some selected *Phallus* species. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Values above the branches are parsimony bootstrap ($\geq 50\%$). The tree is rooted with *Ramaria stricta*.

Phallus mengsongensis H.L. Li, L. Ye, P.E. Mortimer, J.C. Xu & K.D. Hyde, sp. nov. Fig. 2. C-1, C-2.
MycoBank MB 804689

Type:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 4 October 2012, Lei Ye (HKAS 78343, **holotype**).

Basidiocarp egg-shaped at first, elliptical or ovoid, 17–20 mm high \times 9–11 mm wide, greyish white or light grey (1B1 or 1C1) with scales, and short white basal rhizomorphs. The egg soon ruptures to reveal a cap and stipe which reaches a height of 94–142 mm at maturity. Indusium absent. Cap 12–28 mm long, 8–18 mm wide, reddish-orange (7A6), narrowly campanulate with a well-developed apical opening (Fig. 2. C-1). Cap surface reticulated with strongly protruding ridges and sunken pits; the pits of the gleba containing a sticky, smelly, yellowish-brown (5E4) spore mass, which is carried away by flies and then the reddish-orange (7A6) cap is revealed. Stipe 94–142mm

high \times 3–5mm wide, uniformly pure white to pale orange (6A3), erect, pliant, and nearly cylindrical or more usually tapering upwards, slender, hollow, the walls being deeply, and multiply lacunose, the elliptical or polygonal lacunae forming a sort of fractal sponge. Volva present, with light brown (6E8) appressed scales on a greyish-white (1B1) background, with short, white, basal rhizomorphs. Basidiospores [100/4/4] $3.5\text{--}5 \times 1.5\text{--}2\mu\text{m}$, $L=4.1$, $W=1.9$, $Q=2.15$, elongate or cylindrical, smooth and thick walled, hyaline, with oil droplets. Hyphae of cap and stipe $32\text{--}50\mu\text{m}$ wide, foam-like (not tightly packed), variously shaped, hyaline, and smooth-walled. Hyphae of volva $3\text{--}5\mu\text{m}$ wide, branched, hyaline, septate, with clamp connections (Fig. 3). Odour of excrement or carrion.



FIGURE 2. Plate of the *Phallus* species. A1–A2: *Phallus* sp. 1 (HKAS78339), B1: *P. serrata* (HKAS78341), B2: *P. serrata* (HKAS78340), C1–C2: *P. mengsongensis* (HKAS78343). Red arrow in B-1: the serrated margin within holes of indusium of *P. serrata* (HKAS78340). Red circle in C-1: the remarkable well-developed apical opening of *P. mengsongensis* (HKAS78343).

Habitat/Distribution:—gregarious on rotten woods and sticks, Yunnan Province, China.

Etymology:—the species epithet “mengsongensis” refers to the place where the species was collected.

Other material examined:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 20 September 2012, Lei Ye (HKAS 78342, **paratype**); *ibid.* 16 October 2012, Lei Ye (HKAS78345, **paratype**); *Ibid.* 17 October 2012, Lei Ye (HKAS78344, **paratype**).

Notes:—The main distinguishing characters of *Phallus mengsongensis* are a remarkable well-developed opening at the apex of the narrowly campanulate cap (Fig. 2. C-1), and the colour of the cap and stipe, which is reddish-orange (7A6) and pale orange (6A3), respectively. The deeply reticulate stipe and scaly volva are also useful in distinguishing the new species from others in the genus. This species morphologically closely resembles *P. rubicundus* (Bosc) Fr. (1823: 284), *P. calongei* G. Moreno & Khalid (2009: 458), *P. drewesii* Desjardin & B.A.

Perry (2009: 545), *P. minusculus* Kreisel & Calonge (2002: 600), and four varieties of *P. costatus* which are similar in colour. Immature fruiting bodies of *Phallus* species grow underground, are roughly spherical to ovoid, and have a soft or gelatinous surface; the mature fruit bodies are bell-shaped with a reticulated cap, single stipe and volva (Moreno *et al.* 2009; Desjardin & Perry 2009; Calonge 2002). However, *P. mengsongensis*, *P. rubicundus*, *P. calongei*, *P. drewesii*, *P. minusculus* and four varieties of *P. costatus* have subtle morphological differences (Table 2 & 3). If we compare *P. mengsongensis* with *P. calongei*, the cap of *P. calongei* sweeps upwards at the margin, the stipe is white without a deeply reticulate pattern made up of elliptical or polygonal segments, and the volva of *P. calongei* is not scaly (Moreno *et al.* 2009). *P. drewesii* should be compared with *P. mengsongensis*; however the cap of *P. drewesii* is white with an orange tinge under the gleba and is less strongly reticulate than *P. mengsongensis*; the apical opening of *P. drewesii* is perforate and truncated (Fig. 6. A&B); and the stipe of *P. drewesii* is pure white and the spores are pale brown (Desjardin & Perry 2009). *P. minusculus* should be compared with *P. mengsongensis*; however, the stipe of *P. minusculus* is white without a deeply reticulate pattern, and the volva is not scaly (Calonge 2002). *P. rubicundus* should also be compared with *P. mengsongensis*; however, the cap of *P. rubicundus* is not strongly reticulate, and the top of the cap of *P. rubicundus* doesn't have a remarkable well-developed opening. The red stipe of *P. rubicundus* consists of smaller chambers than *P. mengsongensis*, and *P. rubicundus* has a white volva (Liu *et al.* 2005) (Table 2).

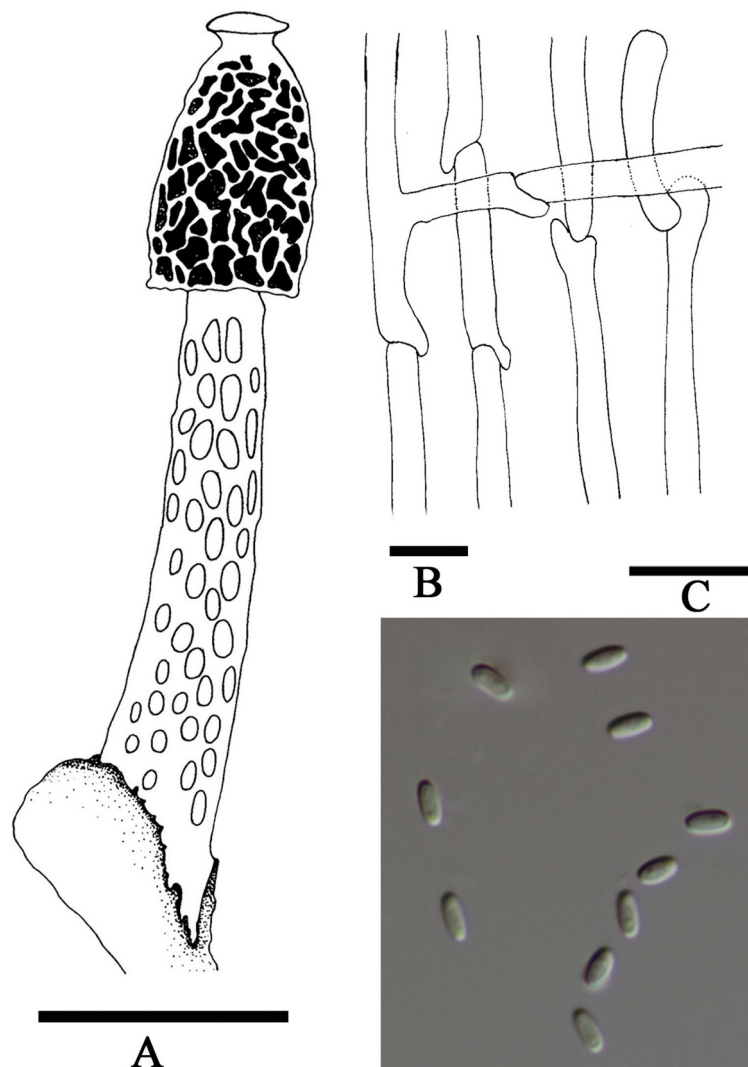


FIGURE 3. Morphological characters of *P. mengsongensis*. A. Basidiocarp B. Hyphae of volva C. Basidiospores. Scale bars: A=3 cm, B=20 μ m, C=10 μ m.

TABLE 2. Synopsis of micro and macro characteristics of *P. mengsongensis*, *P. rubicundus*, *P. calongei*, *P. drewesii* and *P. minusculus*.

Species	<i>P. mengsongensis</i>	<i>P. rubicundus</i>	<i>P. calongei</i>	<i>P. drewesii</i>	<i>P. minusculus</i>
Cap Size (mm)	12–28 × 8–18	16–18 × 15–16	70(28) × 40(14)	7–15 × 5–7	8–10 × 2–5
Cap colour	Orange	Reddish orange	Orange-pink	White	White
Cap surface characters	Strongly reticulate, slimy gleba	Reticulate, slimy gleba	Strongly reticulate, slimy gleba	Reticulate, slimy gleba	Strongly reticulate
Apical Opening characters	See Figure 2: red circle in C-1	Truncated	Truncated	Truncated	Truncated
Stipe shape	Erect or decurved, nearly cylindrical, tapering upwards	Cylindrical or tapering towards the apex or base	Nearly cylindrical	Decurved, cylindrical to subcylindrical, narrowing upward	Cylindrical, attenuate towards the apex
Stipe colour	Light orange	Yellowish orange	White	Pure white	White
Stipe surface characters	Reticulate made up of elliptical segments	Sponge-like	Sponge-like	Deeply reticulate-lacunose with polygonal reticulations	Sponge-like
Volva surface characters	Brown scales	No scales	No scales	Appressed, pubescent to felty scales	No scales
Spore size (µm)	3.5–5 × 1.5–2	3.5–5 × 1.5–2.5	3.5–4.5 × 1.5–2	3–3.5–3.8 × 1.6–2	2–3 × 1–1.5
Spore colour	Hyaline	Hyaline	Hyaline	Pale brown	Hyaline

TABLE 3. Synopsis of macro and micro characteristics of *P. mengsongensis*, *P. costatus* var. *costatus*, *P. costatus* var. *dailingensis*, *P. costatus* var. *epigaeus* and *P. costatus* var. *sphaerocephalus*.

Species	<i>P. mengsongensis</i>	<i>P. costatus</i> var. <i>costatus</i>	<i>P. costatus</i> var. <i>dailingensis</i>	<i>P. costatus</i> var. <i>epigaeus</i>	<i>P. costatus</i> var. <i>sphaerocephalus</i>
Cap size (mm)	12–28 × 8–18	25–40 × 20–30	20–25 × 15–20	25–45 wide	32–40
Cap shape	Narrowly campanulate	Campanulate	Campanulate	Campanulate or cup-like	Spherical to nearly peach-like
Cap colour	Orange	Yellow	Yellow	Light yellow	Yellow
Apical opening characters	See Figure 2: Red circle in C-1	Plate-like	Collar-like, strongly and outwardly incurved	Large plate-like, coarse reticulate veins on the surface	Outwardly incurved top
Stipe shape	Nearly cylindrical, tapering upwards	Cylindrical or tapering upwards	Cylindrical	Cylindrical	Cylindrical or tapering upwards or downwards
Stipe colour	Light orange	Yellow or light yellow to white	Light yellow	Light yellow	Light yellowish brown to light orangish brown
Stipe characters	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Partial veil	Absent	Absent	Present	Absent	Present
Spore size (µm)	3.5–5 × 1.5–2	3.5–4.2 × 1.5–2	3.5–4 × 1.5–2	3.5–3.8 × 1.3–1.6	3.5–4 × 1.5–1.7
Spore colour	Hyaline	Light green	Light green	Light olive green	Light olive green to hyaline

The fruiting body colour of *P. mengsongensis* closely resembles *P. costatus* (Penz) Lloyd var. *costatus* (1909: 10), *P. costatus* (Penz) Lloyd var. *dailingensis* (1954: 72), *P. costatus* (Penz) Lloyd var. *epigaeus* (1938: 76) and *P.*

costatus (Penz) Lloyd var. *sphaerocephalus* (2002: 123). However, the fruiting bodies of *P. costatus* var. *costatus* are larger and yellow, and contain a plate-like apical opening and light green spores; whereas *P. costatus* var. *dailingensis* has a collar-like, outwardly incurved, apical opening, a white partial veil and light green spores. *P. costatus* var. *epigaeus* has a large plate-like apical opening with coarse reticulate veins and light olive green spores, whereas *P. costatus* var. *sphaerocephalus* has a nearly spherical or peach-like and yellow cap, and outwardly incurved apical opening with reticulate veins on surface and white veil (Liu *et al.* 2005) (Table 3).

Phallus serrata H.L. Li, L. Ye, P.E. Mortimer, J.C. Xu & K.D. Hyde, sp. nov. Fig. 2. B-1, B-2.
Mycobank MB 804688

Type:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 21 September 2012, Lei Ye (HKAS78340, **holotype**).

Basidiocarp 150–210 mm high × 15–20 mm wide when mature, subglobose or egg-shaped when young; exoperidium soon rupturing to reveal the cap and stipe on maturity. Cap 40–42 mm high × 34–35 mm wide, nearly half egg-shaped, with a somewhat prominent apex, a white hole, strongly reticulate, white ridges and olive (1E3–5) sticky gleba in the pits. Stipe white, 150–155 mm high × 15–20 mm wide, cylindrical, spongiform. Indusium 135mm–140mm long, white, well-developed, at first contracted under the edge of the cap, later expanding to a skirt-like net whose margin almost touches the ground. The holes within the indusium are round, nearly round, or polygonal, and their edges are serrated (Fig. 2. B-1). Volva brownish-grey (6C2 to 6C4), without scales, attached by a rhizomorph to the soil. Basidiospores [50/2/2] 4–5 × 2–3 μm, L=4.7, W=2.7, Q=1.7, ellipsoid or elongate, hyaline, smooth, and thick walled. Basidia and cystidia not seen. Hyphae of volva 3–5 μm wide, branched, thin-walled, clamp connections present. Hyphae of indusium and stipe foam-like (not tightly packed), with subglobose, hyaline hyphal elements 40–60 μm diameter (Fig. 4). Odour: foul, attractive to flies.

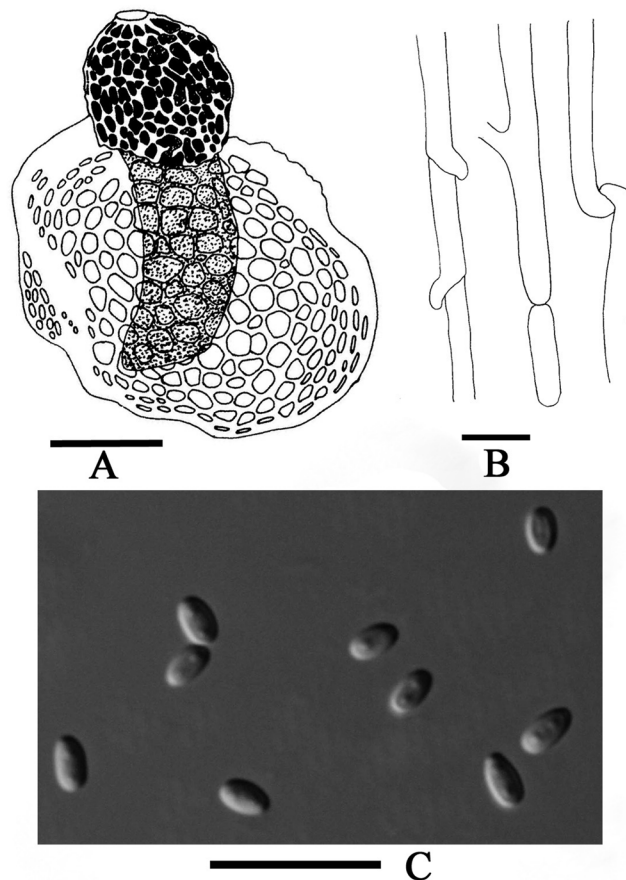


FIGURE 4. Morphological characters of *P. serrata*. A. Basidiocarp B. Hyphae of volva C. Basidiospores; Scale bars: A=5 cm, B=10 μm, C=10 μm.

Habitat/Distribution:—on soil, in decaying litter, solitary or in groups, Yunnan Province, China.

Etymology:—the species epithet “serrata” refers to the serrated edges of the holes within the indusium.

Other material examined:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 8 September 2012, Lei Ye (HKAS78341, **paratype**).

Notes:—The main distinguishing characters of *Phallus serrata* are a white indusium, whose holes are round, nearly round, or polygonal with serrated margins (Fig. 2. B-1), nearly half egg-shaped cap, with a somewhat prominent apex which has a white opening, and white, cylindrical and spongy stipe. This species closely resembles *Phallus indusiatus* Vent. (1798: 520) (*Dictyophora indusiata* (Vent.) Desv. (1809: 92), *Phallus echinolvata* (M. Zang, D.R. Zheng & Z.X. Hu) Kreisel (1996:277), *Dictyophora echinolvata* M. Zang, D.R. Zheng & Z.X. Hu (1988: 146) and *Phallus duplicatus* Bosc (1811: 86) (*Dictyophora duplicata* (Bosc) E. Fisch (1988: 6)). *P. serrata* is comparable with *P. indusiatus* (Cunningham 1944); however, the cap of *P. indusiatus* is campanulate and dingy yellow under the olivaceous gleba, and the indusium is polygonal without serrated margins around the holes. *P. serrata* should also be compared with *P. echinolvata* (Zang *et al.* 1988); however, the cap of *P. echinolvata* is conical or campanulate and whitish yellow under an olivaceous brown gleba, and the indusium is fragile without a serrated margin in the holes, and the volva surface of *P. echinolvata* is echinulate (Table 3). *P. serrata* closely resembles *P. duplicatus* (Liu *et al.* 2005); however, the indusium of *P. duplicatus* is not serrate, and is 60–70mm long, which is shorter than *P. serrata*. The spores of *P. duplicatus* are also smaller than *P. serrata*, and *P. duplicatus* has an annulus under the indusium unlike *P. serrata* (Table 4).

TABLE 4. Synopsis of macro and micro characteristics of *P. serrata*, *P. duplicatus*, *P. indusiatus* and *P. echinolvata*.

Species	<i>P. serrata</i>	<i>P. duplicatus</i>	<i>P. indusiatus</i>	<i>P. echinolvata</i>
Cap shape	Half-egg	Campanulate to conical	Campanulate	Conical or campanulate
Cap colour	White under olive gleba	White under dark brown gleba	Dingy yellow under olivaceous gleba	Whitish yellow under olivaceous brown or dark brown gleba
Cap surface characters	Reticulate, the reticulation being even and with rounded edges	Reticulate, the reticulation being even and with rounded edges	Reticulate-rugulose, the reticulation being even and with rounded edges	Rugose-reticulate, the reticulation being even and with rounded edges
Indusium characters	Round, nearly round or polygon hole with the serrated margin in hole, whose length is 135–140mm	Subglobose to polygonal without the serrated margin in hole, whose length is 60–70mm	Coarsely latticed, apertures large and polygonal without the serrated margin in hole	Fragile, reticulate without the serrated margin in hole
Annulus	Absent	Present	Absent	Absent
Volva characters	Not echinulate	Not echinulate	Not echinulate	Echinulate
Spore shape	Ellipsoid	Cylindrical	Elliptical	Elliptical
Spore size (µm)	4–5×2–3	3.5–4.2 × 1–1.5	3.5–4.5 × 1.5–2	3–4 × 1.3–2
Spore colour	Hyaline	Hyaline	Hyaline	Hyaline or olivaceous-hyaline

Phallus sp. 1. Fig. 2. A-1, A-2.

Basidiocarp 150 mm high × 17 mm wide, spherical to subglobose when young, the exoperidium rupturing at maturity to reveal the cap and stipe. Cap 42 mm × 24 mm, campanulate, with a white opening at apex; surface smooth when young (A1–3), becoming reticulate with irregular ridges and pits, and showing white as flies consume the olive (1–3F5–8) gleba, which is slimy and smelly. Stipe hollow, pure white, 65 mm high × 17 mm wide, cylindrical, and reticulate or lacunose with elliptical or polygonal lacunae. Indusium at first folded up under

the cap, later expanding to become well developed with orange tones. Volva off-white, without scales, but not smooth to the touch, attached to the soil by a thick rhizomorph. Basidiospores [25/1/1] $2.96\text{--}4.93 \times 2\text{--}3 \mu\text{m}$, $L=4.22$, $W=2.05$, $Q=2.06$, ellipsoidal or oblong to cylindrical, hyaline with drops, wall smooth and slightly thickened. Hyphae of stipe hyaline, and foam-like (not tightly packed) under the microscope. Basidia and cystidia not seen. Hyphae of volva hyaline, tubulose, and branched, with clamp connections (Fig. 5). Odour: foul or putrid.

Habitat/Distribution:—on soil, in rotting litter, solitary or in groups, Yunnan Province, China.

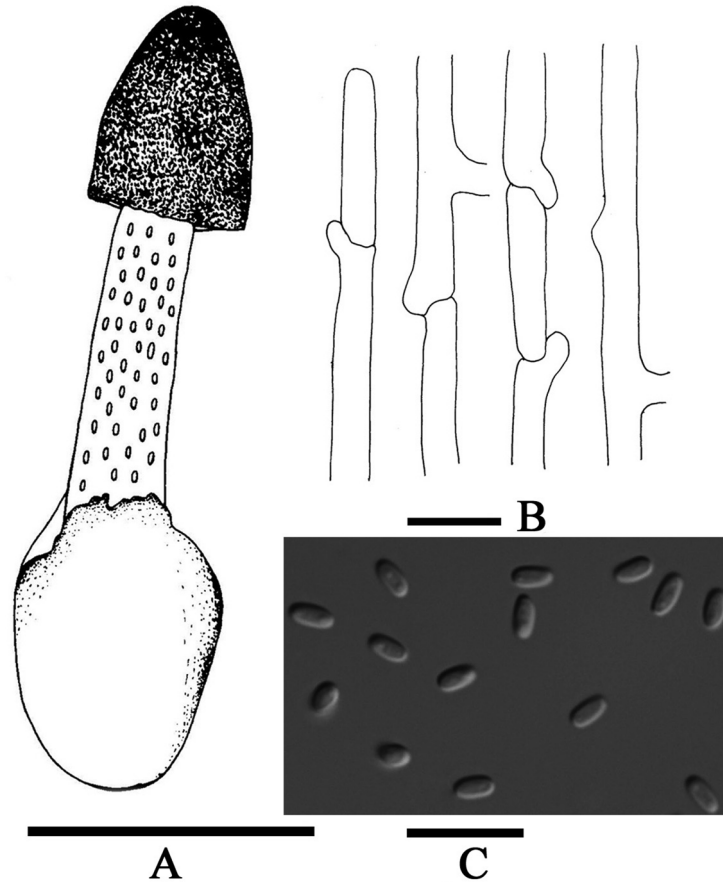


FIGURE 5. Morphological characters of *Phallus* sp. 1. A. Basidiocarp B. Hyphae of volva C. Basidiospores. Scale bars: A=5 cm, B=50 μm , C=10 μm .

Notes:—The main characters of *Phallus* sp. 1 are a slightly orange indusium, which expands from under the cap at maturity and the white cap, smooth without reticulation under the olive (1–3F5–8) gleba (Fig. 2. A-1, A-2 & Fig. 6. C), when immature. Although phylogenetic tree shows 100% bootstrap support for *Phallus* sp. 1 and *P. luteus* (Liou & L. Hwang) T. Kasuya (2009: 106), morphologically *P.* sp. 1 and *P. luteus* are different, even though the specimen of *P.* sp. 1 used in this study is immature. When young, the cap of *P. luteus* is reticulate under the olive brown gleba, the indusium of *P. luteus* is yellow, and the spore size of *P. luteus* is smaller than that of *P.* sp. 1 (Kasuya 2008). *P.* sp. 1 should also be compared with *Phallus multicolor* (Berk. & Broome) Cooke (1882?57); however, the cap surface of *P. multicolor* is lemon yellow under the olive brown gleba and the stipe is yellow white (Dutta *et al.* 2012). As only one specimen was found, we described it, but do not introduce it as a new species in this paper.

Material examined:—CHINA. Yunnan Province: Xishuangbanna, Mengsong Village, UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 14 August 2012, Lei Ye, (HKAS78339).

Discussion

According to the results of the phylogenetic tree, *P. mengsongensis* without indusium and *P. serrata* with indusium

grouped together with 93% bootstrap support. Cabral *et al.* (2012) opined that the indusium cannot be used as a phylogenetically informative character to distinguish genera of *Phallaceae*. Thus, we consider *Dictyophora* as a synonym of *Phallus* irrespective of the presence of an indusium. The morphological characters of *P. mengsongensis* (Table 2 & 3) and *P. serrata* (Table 4), together with the molecular analysis data (Fig. 1) provide strong support that they are distinct new species. We compared our new species with all morphologically and phylogenetically similar taxa (Table 2, 3 & 4) and elucidated *P. mengsongensis* and *P. serrata* as distinct new species. The main distinguishable characteristics between *P. mengsongensis* and *P. serrata* are the indusium, cap colour and surface features, stipe surface features, volva, and basidiospores. *P. serrata* has an indusium with a round, nearly round or polygonal hole, and the hole has a serrated margin (Fig. 2. B-1) whereas *P. mengsongensis* lacks an indusium. The cap of *P. mengsongensis* is orange, has a strongly reticulate and slimy gleba, and has a remarkable well-developed opening at the apex of narrowly campanulate cap (Fig. 2. C-1), whereas the cap of *P. serrata* is white under the olive gleba, and the surface is reticulate with rounded edges. The volva surface of *P. mengsongensis* has brown scales, whereas the volva of *P. serrata* is echinulate or spiky. *P. serrata* also has larger basidiospores ($4\text{--}5 \times 2\text{--}3 \mu\text{m}$) than *P. mengsongensis* ($3.5\text{--}5 \times 1.5\text{--}2 \mu\text{m}$).

Phallus sp. 1 was collected only once in Mengsong, Yunnan and the fruiting body was too young to show all the macro- and micro-characters. According to the phylogenetic analysis, the maximum parsimony tree shows that *Phallus* sp. 1 and *P. luteus* (Liou & L. Hwang) T. Kasuya (2009: 106) (*Dictyophora indusiata* f. *lutea* (Liou & L. Hwang) Kobayasi (1965: 327)) cluster together with 100% bootstrap support. Although molecular analysis shows *Phallus* sp. 1 is closely related to *P. luteus*, they are morphologically different. Since we only collected one immature specimen of this species in 2012, we were not able to confirm its novelty. We hope to collect mature specimens in the near future to confirm whether it is a new species.

The main focus of this paper has been on taxonomy and phylogeny; however, it is worth noting that basidiomes of most *Phallus* species are medicinal, potentially edible and cultivatable (Chang 1989; Lee 2002; Wasser 2002; De Silva *et al.* 2013) and industrialization should be the subject of future research in this genus. Scientific information on wild mushrooms is essential for the introduction of new species for the table (Mortimer *et al.* 2012; Yang *et al.* 2012).



FIGURE 6. Plate of the *Phallus* species. A: *Phallus mengsongensis* (HKAS78343), B: *Phallus drewesii* (photograph source: <http://www.sciencedaily.com/releases/2009/06/090615144215.htm>) and C: *Phallus* sp. 1 (HKAS78339).

Acknowledgements

We are grateful to the CGIAR Research Program 1.2 - Humidtropics: Integrated systems for the humid tropics, and the Center for Mountain Ecosystem Studies, (ICRAF-China) & the Kunming Institute of Botany, Chinese Academy of Science (CAS) for providing the financial support for this study. Thailand Research Fund grant - Taxonomy, Phylogeny and biochemistry of Thai Basidiomycetes (BRG 5580009); the National Research Council of Thailand (NRCT), projects - Taxonomy, Phylogeny and cultivation of *Lentinus* species in northern Thailand (NRCT/55201020007), Mae Fah Luang University project – Taxonomy, Phylogeny and cultivation of *Lentinus* species in northern Thailand (MFU/54 1 01 02 00 48), and Thailand Research Fund grant - Taxonomy, Phylogeny and biochemistry of Thai Basidiomycetes (BRG 5580009) are also thanked for supporting this study. We also thank Phongseun Sysouphanhong for the methods of morphological and molecular experiments and data analysis, Jiayu Guo, Michael Pilkington and Lei Ye for sample collecting & Jiankui Liu and Sajeewa Maharachchikumbura for their help in phylogenetic analysis.

References

- Arora, D. (1986) *Mushrooms Demystified*, Ten Speed Press, pp 1056.
- Cabral, T.S., Marinho, P., Goto, B.T. & Baseia, I.G. (2012) *Abrachium*, a new genus in the Clathraceae, and *Itajahya* reassessed. *Mycotaxon* 119: 419–429.
<http://dx.doi.org/10.5248/119.419>
- Calonge, F.D. (2002) *Phallus minusculus* sp. nova from Tropical Africa. *Feddes Repertorium* 113: 600–602.
- Chang, S.T. & Miles, P.G. (1989) *Edible mushrooms and their cultivation*, ISBN 0-8493-6758-X, pp 345.
- Cunningham, G.H. (1944) *Gasteromycetes of Australia and New Zealand*. Dunedin, McIndoe, pp 236.
- Dai, Y.C., Yang, Z.L., Wen, H.A., Bau, T. & Li, T.H. (2010) A revised checklist of edible fungi in China. *Mycosystema* 29(1): 1–21.
- Desjardin, D.E. & Perry, B.A. (2009) A new species of *Phallus* from São Tomé, Africa. *Mycologia* 101(4): 545–547.
<http://dx.doi.org/10.3852/08-166>
- De Silva, D.D., Rapior, S., Sudarman, E., Stadler M., Xu, J., Alias, S.A. & Hyde, K.D., Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry, *Fungal Diversity*, 2013; 62: 1–40.
<http://dx.doi.org/10.1007/s13225-013-0265-2>
- Douanla-Meli, C., Langer, E. & Calonge, F.D. (2005) *Geastrum pleosporus* sp. nov., a new species of Geastraceae identified by morphological and molecular phylogenetic data. *Mycological Progress* 4(3): 239–250.
<http://dx.doi.org/10.1007/s11557-006-0127-3>
- Dutta A.K., Chakraborty N., Pradhan P. & Acharya K. (2012) Phallales of West Bengal, India. II. Phallaceae: *Phallus* and *Mutinus*. *Researcher* 4(8): 21–25.
- Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2): 113–118.
<http://dx.doi.org/10.1111/j.1365-294x.1993.tb00005.x>
- Huang, Y. (1993) Biological characteristics of *Dictyophora rubrovalvata*. *Journal of Zhejiang University (Agriculture & Life Science)* 19(2): 183–187.
- Index Fungorum (2013) Available from: <http://www.indexfungorum.org/names> (accessed 17 December 2013).
- Kasuya, T. (2008) *Phallus luteus* comb. nov., a new taxonomic treatment of a tropical phalloid fungus. *Mycotaxon* 106: 7–13
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008) *Ainsworth and Bisby's Dictionary of the Fungi*, 10th Edition, CABI, Europe, UK.
- Kornerup, A. & Wanscher, J.H. (1978) *Methuen handbook of colour*. Eyre Methuen, London, pp 252.
- Kreisel, H. (1996) A preliminary survey of the genus *Phallus* sensu lato. *Czech Mycology* 48(4): 273–281.
- Lee, I.K., Yun, B.S., Han, G., Cho, D.H., Kim, Y.H. & Yoo, I.D. (2002) Dictyoquinazols A, B, and C, new neuroprotective compounds from the mushroom *Dictyophora indusiata*. *Journal of Natural Products* 65: 1769–1772.
- Li, T.H., Wu, X.L., Liu, B. & Deng, W.Q. (2004) A study on Phallaceae from Yunnan, Guizhou and Guangxi, China. *Guizhou Science* 22: 80–89
- Lin, C., Lin, R. & Lin, X. (2011) Study on the development of the comprehensive utilization about the *Dictyophora* resource. *Edible Fungi of China* 30(2): 8–11.
- Liu, B., Fan, L., Li, J.Z., Li, T.H., Song, B. & Li, J.H. (2005) *Flora Fungorum Sinicorum* Vol. 23, Beijing: Science Press, pp 137–171.
- Lu, D. & Luo, C. (2010) A Study on the Biological Characteristic of *Phallus impudicus*. *Journal of Xinyang Normal University Natural Science* 23(2): 242–244.
- Moreno, G., Khalid, A.N. & Alvarado, P. (2009) A new species of *Phallus* from Pakistan. *Mycotaxon* 108(1): 457–462.

<http://dx.doi.org/10.5248/108.457>

- Mortimer, P.E., Karunarathna, S.C., Li, Q.H., Gui, H., Yang, X.F., Yang, X.Q., He, J., Ye, L., Guo, J.Y., Li, H.L., Sysouphanthong, P., Zhou, D.Q., Xu, J.C. & Hyde, K.D. (2012) Prized edible Asian mushrooms: ecology, conservation and sustainability. *Fungal Diversity* 56(1): 31–47.
<http://dx.doi.org/10.1007/s13225-012-0196-3>
- Wasser, S.P. (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology* 60: 258–274.
<http://dx.doi.org/10.1007/s00253-002-1076-7>
- Yang, X.F., Luedeling, E., Chen, G.L., Hyde, K.D., Yang, Y.J., Zhou, D.Q., Xu, J.C. & Yang, Y.P. (2012) Climate change effects fruiting of the prize matsutake mushroom in China. *Fungal Diversity* 56:189–198.
<http://dx.doi.org/10.1007/s13225-012-0163-z>
- Yang, Z.L. & Ge, Z.W. (2008) *Phallogaster saccatus* (Basidiomycetes, Fungi), First record from East Asia. *Acta Botanica Yunnanica* 30(2): 147–150.
- Zang, M., Zheng, D.R. & Hu, Z.X. (1988) A new species of the genus *Dictyophora* from China. *Mycotaxon* 33: 145–148.