diversity

MDPI

Article

Three New Species of *Cystolepiota* from Laos and Thailand

Phongeun Sysouphanthong [1,2,3], Naritsada Thongklang [1,2,*], Yuan S. Liu [4] and Else C. Vellinga [5]

[1] Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand; laofungi@gmail.com
[2] School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
[3] Ecology Division, Biotechnology and Ecology Institute, Ministry of Forestry and Agriculture, Vientiane P.O. Box 811, Laos
[4] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand; yuanshuailiu9@gmail.com
[5] UC Herbarium, UC Berkeley, Berkeley, CA 94720-2465, USA; ecvellinga@comcast.net
* Correspondence: naritsada.t@gmail.com; Tel.: +66-539-16996

Abstract: *Cystolepiota* Singer is rarely studied in Southeast Asia; here, we survey and describe three new species of *Cystolepiota* from tropical Laos and Thailand. *Cystolepiota pyramidalis* is related to *C. fumosifolia* (Murrill) Vellinga and *C. pseudofumosifolia* M.L. Xu & R.L. Zhao, but it is distinguished by pale to pastel yellow lamellae. Second, *Cystolepiota thailandica* differs from other members in the genus by the greyish-orange granulose or powdery pileus and stipe covering made up of globose to subglobose and sphaeropedunculate elements. Furthermore, *Cystolepiota rhodella* is characterized by the pink-violet granulose covering of the pileus and stipe and white lamellae with distinctly violet edges. Each species is provided with a full description of the morphological characters, photos in situ, line drawings of the microcharacters, discussion of related and similar species, and molecular data.

Keywords: Agaricaceae; diversity; lepiotaceous fungi; new species; phylogeny; Southeast Asia; taxonomy

check for updates

Citation: Sysouphanthong, P.; Thongklang, N.; Liu, Y.S.; Vellinga, E.C. Three New Species of *Cystolepiota* from Laos and Thailand. *Diversity* **2022**, *14*, 449. https://doi.org/10.3390/d14060449

Academic Editor: Michael Wink

Received: 4 April 2022
Accepted: 31 May 2022
Published: 2 June 2022

1. Introduction

The genus *Cystolepiota* Singer (Agaricaceae s.l.) is very diverse, and all species are saprotrophic. The estimates of the number of species in the genus range from twelve [1] to forty-five [2]. The species are characterized by small pluteoid or lepiotoid basidiomata with a granulose to powdery covering, a white to pale lilac spore print, and the hyaline, non-dextrinoid, or dextrinoid spores that can be smooth or slightly roughened, with or without cheilocystidia and pleurocystidia, a regular hymenophoral trama, the epitheloid pileus and stipe covering made up of globose, broadly ellipsoid to elongated elements, and the absence or presence of clamp connections. The species with elongated elements in the pileus covering and bi-nucleate spores were placed in the separate genus *Pulverolepiota* [3]; this genus was later considered a section of *Cystolepiota*. The three sections in the genus, viz., sect. *Cystolepiota* Singer, sect. *Pulverolepiota* (M. Bon) Vellinga, and sect. *Pseudoamyloideae* Singer & Clém. are distinguished based on morphological characters such as the shape of the pileus covering elements (elongated and irregular in sect. *Pulverolepiota*) and the reaction of the spore wall in Melzer's reagent (dextrinoid in sect. *Pseudoamyloideae*) [4]. Species in sect. *Pulverolepiota* also differ in the binucleate spores, whereas the other species have uninucleate spores.

Melanophyllum Velen. species share many characters with *Cystolepiota* but differ in the colored, ornamented spores. Molecular phylogenetic studies [5,6] showed that *Melanophyllum* and *Cystolepiota* together form a monophyletic group; *Melanophyllum* is the oldest name available for this group, but there are no sequence data available for its type, *M. canali* Velen.; *Fusispora* Fay., typified by species *L. sistrata* Quél. Fr., which is generally considered

to belong to *Cystolepiota,* is considered a nomen dubium by Donk [7], as the spore sizes given are about 16 × 5 µm [8], a size not found in any *Cystolepiota* species.

Some authors, e.g., Knudsen [9] and Bon [10] considered *Lepiota* sect. *Echinatae*, also characterized by globose to ellipsoid elements in the pileus covering, part of *Cystolepiota*; in 1980, Knudsen [9] came back from that idea and placed those species again in *Lepiota*, but Bon [11] erected a separate genus, *Echinoderma* (Locq. ex Bon) Bon, for those species. Recently, Vellinga [12] and Hou and Ge [6] showed that, based on molecular characters, those *Echinoderma* species with small ellipsoid spores still belong in *Lepiota* and that the species, such as *E. asperum* (Pers.) Bon, with elongated spores are separate from both *Lepiota* and *Cystolepiota* and are placed in *Echinoderma*.

Cystolepiota is rarely studied in Laos and Thailand. Only *Cystolepiota seminuda* (Lasch) Bon and *Cystolepiota sistrata* (Fr.) Singer ex Bon and Bellù were recorded in Thailand [13]. Sysouphanthong et al. [14] reported *C.* aff. *icterina* F.H. Møller ex Knudsen, and *C. pulverulenta* (Huijsman) Vellinga from Laos. However, the records of those species were only based on their morphology. In this study, three new species are examined from Laos and Thailand; details of their morphology, ecology, and distribution and a molecular phylogenetic analysis are presented.

2. Materials and Methods

2.1. Collecting and Material Examination

During the rainy seasons of 2012–2018, samples were collected in Chiang Mai and Chiang Rai Provinces (Northern Thailand) and Oudomxay Province (Northern Laos). Thai specimens were deposited in the Herbarium of Mae Fah Luang University (MFLU), and Lao specimens were deposited in the National Herbarium of Laos (HNL). Fresh samples were photographed in situ, and the ecology of the original places was recorded. After collection, macro- and microscopic characters of the specimens were observed in the laboratory. The main features of fresh samples are pileus, lamellae, stipe, annulus, context, spore print, taste, and odor. Color features were described and followed the codes of Kornerup and Wanscher [15]. After macroscopic observation, specimens were dried in a hot air dryer around 30–40 °C for 24 h and deposited to preserve in the fungarium for future studies. Microscopical characteristics were observed and illustrated from fresh or dry specimens with a compound microscope. Distilled water and 3–10% KOH were used to observe color features; Melzer's reagent, cotton blue, and cresyl blue were used to examine spore reaction, and ammoniacal Congo red was used to stain spore walls and hyphae. Spore measurements were made from 25 spores of each basidioma of each collection. The terminology of the features followed Vellinga and Noordeloos [4]. The following abbreviations are used: "avl" for average length, "avw" for average width, "Q" for quotient of length and width, and "avQ" for average quotient.

2.2. Phylogenetic Study

DNA was extracted from dried collections according to the instructions of the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, China). For the PCR and PCR amplification, primers ITS1/ITS4 or ITS1-F/ITS4 were used for the nrITS1, 5.8S, and nrITS2 regions [16,17], primers LR0R/LR5 for the large subunit region (LSU) [18,19], and primers fRPB2-5C/fRPB2-7CR for the polymerase II second largest subunit (RPB2) region [20]. Sequencing was performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. The sequences were checked and assembled using the SeqMan program (DNAStar, Madison, WI, USA), and new sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/ (accessed on 1 June 2022)).

Available sequences of *Cystolepiota,* representatives of *Lepiota,* and of the closely related genera *Echinoderma*, *Melanophyllum,* and *Smithiomyces* were obtained from GenBank. Each dataset was first aligned using MAFFT version 7.130-win32 [21,22]. The final dataset comprised 82 collections and 2411 characters (including gaps), which were 78 collections and 751 characters from ITS, 51 collections and 881 characters from LSU, and 35 collections

and 779 characters for RPB2. The final alignments were submitted to TreeBASE (ID: 29388). A maximum-likelihood (ML) analysis was performed using RAxML version 7.2.6 [23] with GTRGAMMAI as the model of evolution, and branch support was estimated over 1000 bootstrap partitions (BP) with the rapid bootstrap option. A Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 [24]. The best substitution model of individual genes was determined using MrModelTest v.2.3 [25]. The best selected model (GTR+I+G) was for ITS, LSU, and RPB2. For the BI analysis setting, three Metropolis-coupled Markov chain Monte Carlo (MCMC) runs, each with four heated chains and two cold chains, and the run was conducted for 5 million generations and sampled every 1000 generations, with the first 10% discarded as burn-in. The phylogram results of all analyses were exported and edited in TreeView 1.0.0.0 [26]. The phylograms were edited in the software of Adobe Illustrator CS3.

3. Results and Discussion

3.1. Phylogeny

The maximum-likelihood phylogram of representative lepiotoid mushrooms was based on a multi-gene DNA dataset made up of ITS, LSU, and RPB2 gene regions (Table 1). The alignment comprised 74 specimens with 2103 characters in total (including the gaps). The best RaxML phylogram, with a final likelihood value of −23,323.039705, is presented. The matrix had 1151 distinct alignment patterns with 38.63% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.253717, C = 0.213111, G = 0.263577, and T = 0.269595; substitution rates, AC = 1.484055, AG = 4.134055, AT = 1.770416, CG = 0.419899, CT = 6.554890, and GT = 1.000000; gamma distribution shape parameter, α = 0.929050. The phylogram topology derived from the Bayesian analysis was similar to that derived from the ML analysis. Bootstrap values of ML $\geq$ 70% and bootstrap values of BI $\geq$ 0.95 are indicated in Figure 1.

Table 1. GenBank accession numbers, geographical origins, and voucher numbers of taxa used for the phylogenetic analysis.

Taxon	Country	Voucher Number	GenBank Accession Number		
			ITS	LSU	RPB2
Agaricus campestris	China	LAPAG370	KM657927	KR006607	KT951556
Agaricus friesianus	China	ZRL2012601	KX657026	KX656970	KX685048
Cystolepiota adulterina	Italy	475	JF907978	-	-
Cystolepiota bucknallii	The Netherlands	ecv1761	AY176458	-	-
Cystolepiota bucknallii	Italy	490	JF907979	-	-
Cystolepiota cystidiosa	USA	MICH18884	U85333	U85298	-
Cystolepiota cystophora	Costa Rica	DUKE-JJ87	U85332	U85297	-
Cystolepiota fumosifolia	USA	ecv3278	EF121817	-	-
Cystolepiota hetieri	The Netherlands	ecv2237	AY176459	-	-
Cystolepiota hetieri	Italy	782	JF907982	-	-
Cystolepiota hetieri	China	420526MF0093	MG694259	-	-
Cystolepiota hetieri	Canada	HRL0772	MH979434	-	-
Cystolepiota hetieri	Canada	HRL1277	MH979438	-	-
Cystolepiota hetieri	USA	HRL2162	MH979463	-	-
Cystolepiota hetieri	China	HKAS 84189	MN810139	MN810094	MN820976
Cystolepiota hetieri	China	HKAS 53554	MN810143	MN810102	MN820977
Cystolepiota luteohemisphaerica	Ecuador	TL_11724	AM946477	AM946476	-
Cystolepiota pseudofumosifolia	China	HKAS 104303	MN810150	MN810095	MN820973
Cystolepiota pseudofumosifolia	China	HKAS 105918	MN810152	MN810108	MN820974
Cystolepiota pulverulenta	USA	ecv1872	AF391036	AY176349	-
Cystolepiota pulverulenta	USA	ecv1763	AF391037	-	-
Cystolepiota pyramidalis	**Laos**	**HNL502500**	**MZ574554**	**MZ569511**	**-**
Cystolepiota pyramidalis	**Thailand**	**MFLU 12-1774**	**MZ574555**	**MZ569512**	**-**

Table 1. *Cont.*

Taxon	Country	Voucher Number	GenBank Accession Number		
			ITS	LSU	RPB2
Cystolepiota rosea	Italy	781	JF907981	-	-
Cystolepiota seminuda	The Netherlands	H.A. Huijser s.n.	AY176350	AY176351	-
Cystolepiota seminuda	Italy	9247	JF907983	-	-
Cystolepiota seminuda	USA	Smith 2018	MK573889	-	-
Cystolepiota aff. *seminuda*	China	HKAS 73969	MN810144	MN810100	MN820979
Cystolepiota aff. *seminuda*	China	HKAS 92275	MN810149	MN810101	MN820980
Cystolepiota sistrata	Canada	HRL1282	MH979429	-	-
Cystolepiota sistrata	Italy	491	JF907980	-	-
Cystolepiota sistrata	USA	HRL2161	MH979462	-	-
Cystolepiota sp.	China	ZRL2011054	KF804000	-	-
Cystolepiota sp.	China	ZRL2012038	KF804001	-	-
Cystolepiota sp.	Canada	UBC_F24523	MF955171	-	-
Cystolepiota sp.	USA	HRL1900	MH979456	-	-
Cystolepiota sp.	USA	MycoMap_7788	MK560110	-	-
Cystolepiota sp.	USA	MycoMap_7792	MK560111	-	-
Cystolepiota sp.	China	HKAS 78850	MN810142	MN810103	MN820978
Cystolepiota sp.	China	HKAS 105719	MN810151	MN810109	MN820975
Cystolepiota sp. *(Echinoderma)*	Thailand	ecv3896	-	HM488789	-
Cystolepiota sp. *(Echinoderma)*	Malaysia	LAM 0001	-	KY090841	-
Cystolepiota sp.	India	HATFD14_95	KU847887	-	-
Cystolepiota thailandica	**Thailand**	**MFLU 22-0017**	**MZ574556**	**MZ569513**	**-**
Cystolepiota rhodella	**Laos**	**HNL501799**	**MZ574551**	**MZ569508**	**MZ508496**
Cystolepiota rhodella	**Thailand**	**MFLU 22-0019**	**MZ574552**	**MZ569509**	**MZ574090**
Cystolepiota rhodella	**Thailand**	**MFLU 09-0050**	**MZ574553**	**MZ569510**	**-**
Echinoderma asperum	North Macedonia	HKAS 106783	MN810133	MN810088	MN820967
Echinoderma asperum	USA	HKAS 84214	MN810135	MN810089	MN820964
Echinoderma asperum	USA	HKAS 84240	MN810136	MN810090	MN820965
Echinoderma asperum	China	HKAS 106782	MN810134	MN810087	MN820962
Echinoderma asperum	China	HKAS 105694	MN810153	MN810106	MN820966
Echinoderma asperum	China	HKAS 77440	MN810145	MN810096	MN820963
Echinoderma asperum	India	NEHU.MBSRJ.55	KP843884	MG253012	-
Echinoderma flavidoasperum	China	HKAS 87905	MN810147	MN810098	MN820969
Echinoderma flavidoasperum	China	HKAS 76527	MN810146	MN810097	MN820968
Echinoderma hystrix	France	H.A. Huijser	AY176377	AY176378	-
Echinoderma sp.	China	HKAS 70488	MN810148	MN810099	MN820970
Echinoderma sp.	China	HKAS 106735	MN810154	MN810107	MN820971
Lepiota aff. *carinii*	Hungary	NL-2202	-	MK277953	-
Lepiota alba	China	HKAS 90371	MN810115	MN810075	MN820946
Lepiota asperula	Canada	S.D.Russell HRL1281	MH979440	-	-
Lepiota castanea	China	HKAS.84179	MN810119	MN810077	MN820960
Lepiota clypeolaria	China	HKAS 87248	MN810123	MN810080	MN820941
Lepiota echinacea	China	HKAS 105582	MN810155	MN810104	MN820954
Lepiota geocarpa	USA	UTC00143916	HQ020412	EU130550	MN820945
Lepiota geophana	USA	UTC00253060	HQ020411	HQ020421	MN820944
Lepiota jacobi	China	HKAS 48802	MN810138	GU199356	MN820953
Lepiota magnispora	China	HKAS 61622	JN944089	JN940285	JN993693
Lepiota omninoflava	China	HKAS 106734	MN810157	MN810092	MN820951
Melanophyllum eyrei	South Korea	ASIS23988	KF953546	-	-
Melanophyllum eyrei	Sweden	TL6692	AY176493	-	-
Melanophyllum haematospermum	USA	ecv2517	AF391039	AY176456	-
Melanophyllum haematospermum	The Netherlands	ecv2249	AF391038	AY176455	-
Melanophyllum haematospermum	Canada	S.D.Russell HRL1807	MH979452	-	-
Melanophyllum haematospermum	Italy	913	JF908498	-	-

Table 1. *Cont.*

Taxon	Country	Voucher Number	GenBank Accession Number		
			ITS	LSU	RPB2
Smithiomyces asiaticus	China	HKAS 84395	MW522986	MW716269	MW736566
Smithiomyces dominicanus	Dominican Republic	BSD126144	KR604686	MW716266	MW736563
Smithiomyces heterosporus	China	HKAS 84392	MW522985	MW716268	MW736565
Smithiomyces lepiotoides	China	HKAS 54390	MW522984	MW716270	-
Smithiomyces mexicanus	Switzerland	UTC259587	MW723225	MW716267	MW736564

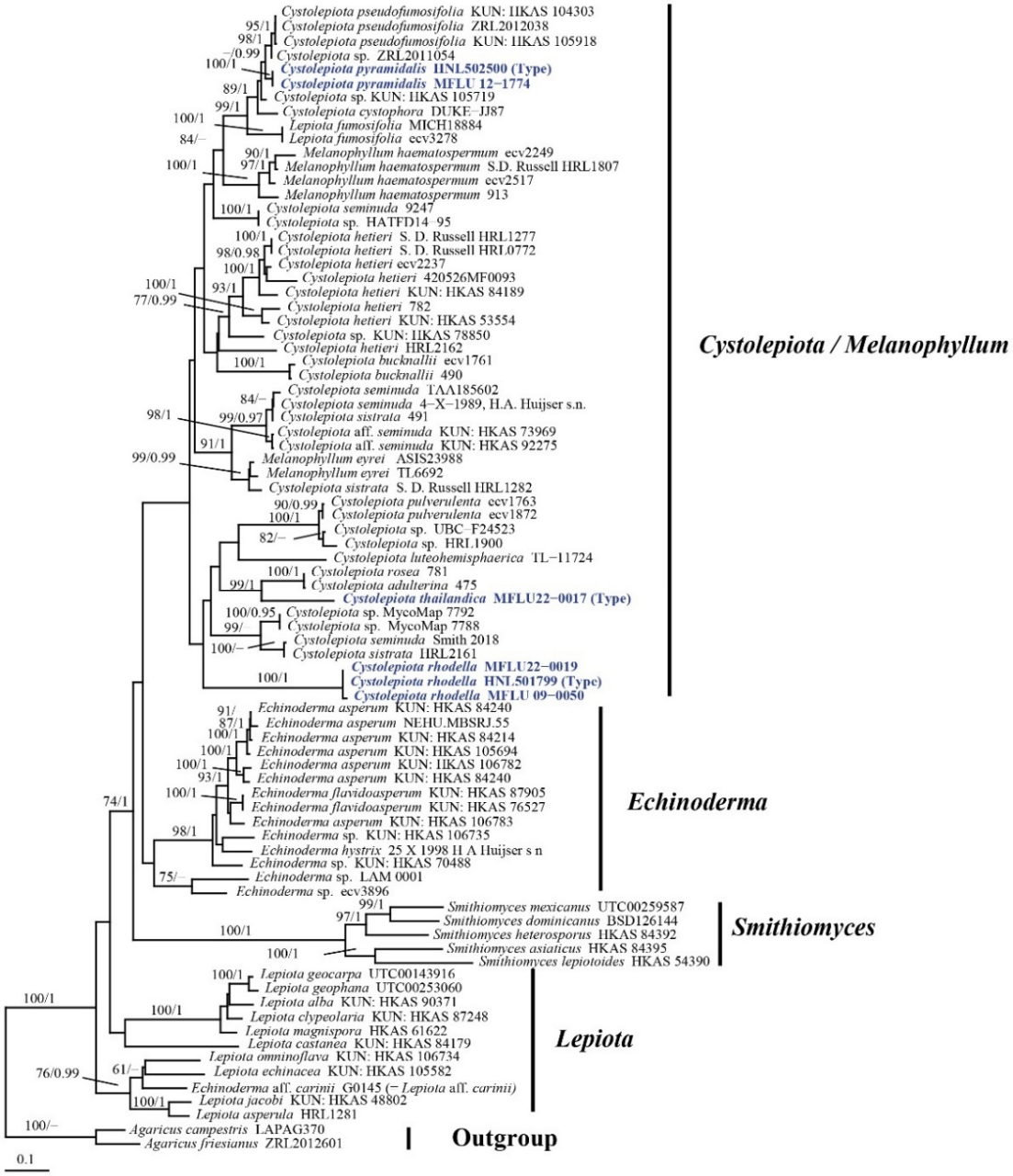

Figure 1. Maximum-likelihood phylogram of *Cystolepiota* and *Melanophyllum* specimens based on combined nrITS-LSU and RPB2 sequences. New sequences generated for this study are in blue. Bootstrap values of ML $\geq$ 60%, and BI $\geq$ 0.95 are indicated above the branches (ML/BI). *Agaricus campestris* L. and *Agaricus friesianus* L.A. Parra, Olariaga & Callac are chosen as outgroup.

The maximum-likelihood phylogram (Figure 1) shows three major clades: a clade of *Cystolepiota* and *Melanophyllum* species, *Echinoderma*, and a clade of *Lepiota* species.

The phylogram in Figure 1 shows clearly that much more work on the genus and its species has to be conducted; many species concepts (e.g., of *C. seminuda* and *C. hetieri*) are not settled yet. A second conclusion is that the morphology-based sections are not reflected in the tree based on molecular characters. The three Thai/Laotian species fall in different clades, with *C. pyramidalis* close to *C. pseudofumosifolia* and *C. fumosifolia*; *C. rhodella* takes a rather isolated position on a long branch basal to the *C. hetieri* complex. The third species, *C. thailandica*, is closely related to species in the *C. seminuda* complex.

3.2. Taxonomy

3.2.1. *Cystolepiota pyramidalis* Sysoup. and Thongkl. sp. nov.

MycoBank: MB843144; FoF number: FoF 10598; Figures 2 and 3.

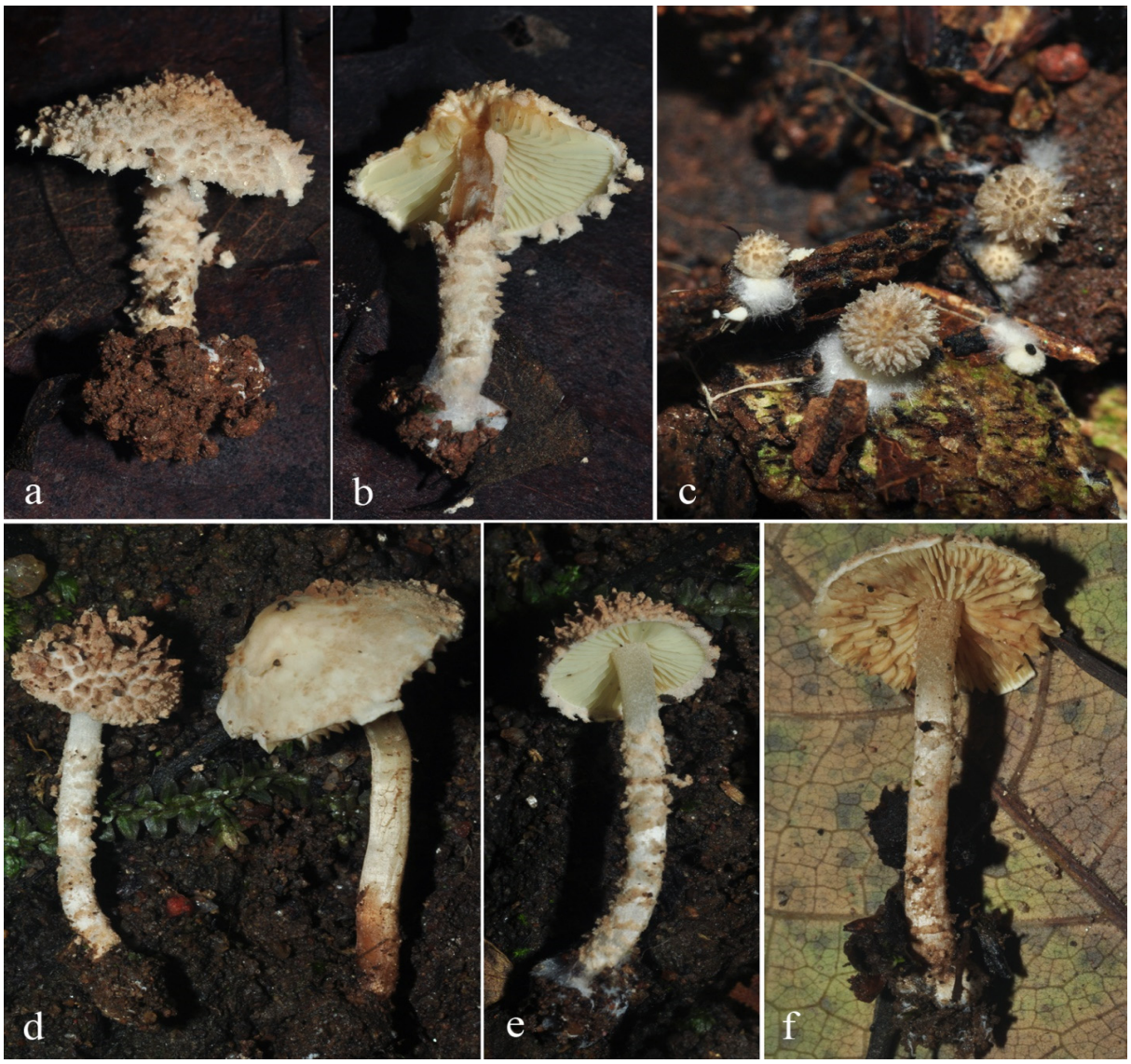

Figure 2. Macrocharacters of *Cystolepiota pyramidalis*. (**a**–**c**) = MFLU12-1774 (paratype), (**d**–**f**) = HNL 502500 (holotype).

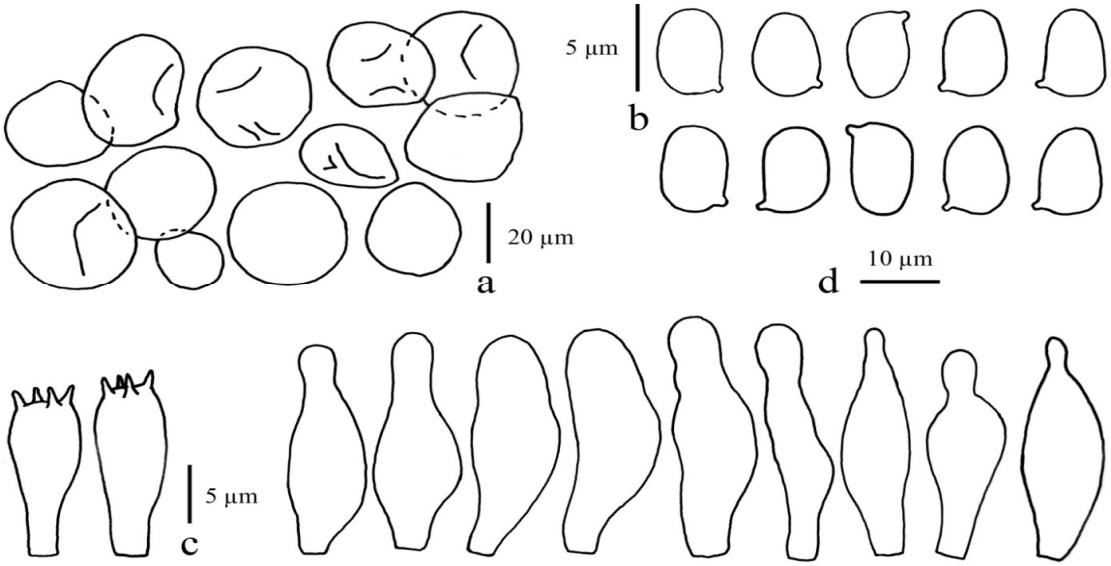

Figure 3. Microcharacters of *Cystolepiota pyramidalis* (HNL502500, holotype). (**a**) = elements of pileus covering, (**b**) = basidiospores, (**c**) = basidia, (**d**) = cheilocystidia.

Etymology—the name "*pyramidalis*" refers to the pyramidal shape of the squamules on the pileus.

Diagnosis—*C. pyramidalis* is recognized by basidiomata covered with light brown to brown pyramidal or irregular pyramidal squamules, pale yellow lamellae, hyaline and ellipsoid-ovoid, smooth basidiospores, variably shaped cheilocystidia with or without excrescence at the apex, the absence of pleurocystidia, epitheliod pileus and stipe covering, and the presence of clamp connections.

Holotype—Laos, Oudomxay Province, Xay District, Houay Houm Village, 18 August 2014, P. Sysouphanthong, PS2014-8290 (HNL502500).

Pileus 15–45 mm diam., first parabolic or campanulate, expanding to convex or umbonate, with straight margin, when young completely covered with crowded pyramidal or irregular pyramidal squamules, brownish (6D7–8), soon breaking up into brownish (6D7–8) to light brown (6D4–5) pyramidal to granular pyramidal squamules or warts toward margin, sparse or fragile when mature, on orange-white to pale orange (5A2–3) background; margin covered with concolorous pyramidal to granular pyramidal velar remnants. Lamellae free, 3–5 mm wide, pale yellow (3A3), becoming brownish orange (6C4–6) when touched or mature, broadly ventricose, with 3–4 lamellulae, with concolorous smooth to slightly eroded edge. Stipe 25–40 × 4–5.5 mm, cylindrical, covered with concolorous squamules to those on pileus, sparse at apex, fragile when mature, on orange-white to pale orange (5A2–3) background. Annulus an annular zone with velar remnants and concolorous to pileus margin, sometimes fragile with age. Context white in pileus, up to 1 mm thick at center; hollow in stipe and concolorous with surface. Odor and taste not observed. Spore pint white.

Basidiospores (50,2,2) 3.8–4.5 × 2.5–3.2 µm, avl × avw = 4.1 × 3.0 µm, Q = 1.25–1.6, avQ = 1.37, ellipsoid-ovoid in frontal view, ellipsoid in side-view, slightly thick-walled, smooth, hyaline, non-dextrinoid, non-amyloid, cyanophilous. Basidia 15–18 × 4.5–7 µm, clavate, thin-walled, hyaline, four-spored, sometimes two-spored. Cheilocystidia 20–40 × 7–15 µm, variable in shape, irregular cylindrical, fusiform, narrowly utriform to utriform, lageniform, clavate with a narrowed apex, slightly thick-walled, hyaline. Pleurocystidia absent. Pileus covering an irregular epithelium composed of globose to subglobose elements, 35–65 µm in diam., slightly thick-walled, smooth, with pale brown to brown parietal and intracellular pigments. Stipe covering an irregular epithelium same as on pileus. Clamp connections present.

Habitat and habit—growing in small groups, saprotrophic on humus-rich soil of mixed deciduous forest with *Castanopsis* spp. and *Lithocarpus* spp. dominant; the species is rare and so far known from Oudomxay province, northern Laos, and Chiang Rai province, northern Thailand.

Additional material examined—Thailand, Chiang Rai Province, Muang District, Phoo Kham Fah Village, 15 August 2012, P. Sysouphanthong, PS2012-11 (MFLU12-1774, paratype).

Notes—*Cystolepiota pyramidalis* is rare in Laos and Thailand; it was found in two locations, not far apart from each other at similar elevations, viz., Oudomxay Province of northern Laos and Chiang Rai Province of northern Thailand. The specimens from these two locations are identical both in morphological and in molecular characters. It is distinguished by light brown to brown pyramidal squamules on basidiomata and pale yellow lamellae. This new species is distinguished from other species by yellow lamellae and the distinct pyramidal shape of the squamules on the pileus.

Cystolepiota pyramidalis belongs to a clade of similar species (Figure 1). *C. fumosifolia*, known from North America and Europe (as *C. cystidiosa* (A.H. Smith) Bon, *C. luteicystidiata* (D.A. Reid) Bon, and *L. lycoperdoides* Kreisel), also has pyramidal granular warts on the pileus, but the cheilocystidia and abundant pleurocystidia have yellow contents, and the cheilocystidia are covered with yellow exudate. *C. pseudofumosifolia* from China has white lamellae and lacks the pyramidal warts on the cap. It lacks pleurocystidia, just like *C. pyramidalis*.

There are some other species with yellow lamellae in the genus. First, *Cystolepiota bucknallii* (Berk. and Broome) Singer & Clémençon, known from temperate regions in Europe and North America, has pale yellow to pastel yellow lamellae, but the pileus and stipe are covered with lilac granulose squamules, and the much longer (7–9 μm) spores are dextrinoid [27,28]. Second, *Cystolepiota seminuda* (Lasch) M. Bon also has white to yellowish creamy lamellae with a pale lemon-yellow tinge, but its basidiomata are much smaller and have a white to cream densely floccose-verrucose covering on the pileus, and it lacks cheilocystidia [28,29].

Additionally, *Cystolepiota oliveirae* P. Roux, M. Paraíso, J.-P. Maurice, A.-C. Normand & F. Fouchier, described from Portugal, has distinctly white to reddish-brown squamules or warts on the pileus and stipe, but the species has white to cream lamellae and rough basidiospores [30], while *C. pyramidalis* has yellow lamellae and smooth basidiospores.

3.2.2. *Cystolepiota thailandica* Yuan S. Liu, Sysouph. and Thongkl. sp. nov.

MycoBank: MB843145; FoF number: FoF 10597; Figures 4 and 5.

Figure 4. Macrocharacters of *Cystolepiota thailandica*. (**a**,**b**) = MFLU22-0017 (holotype), (**c**,**d**) = MFLU22-0018 (paratype).

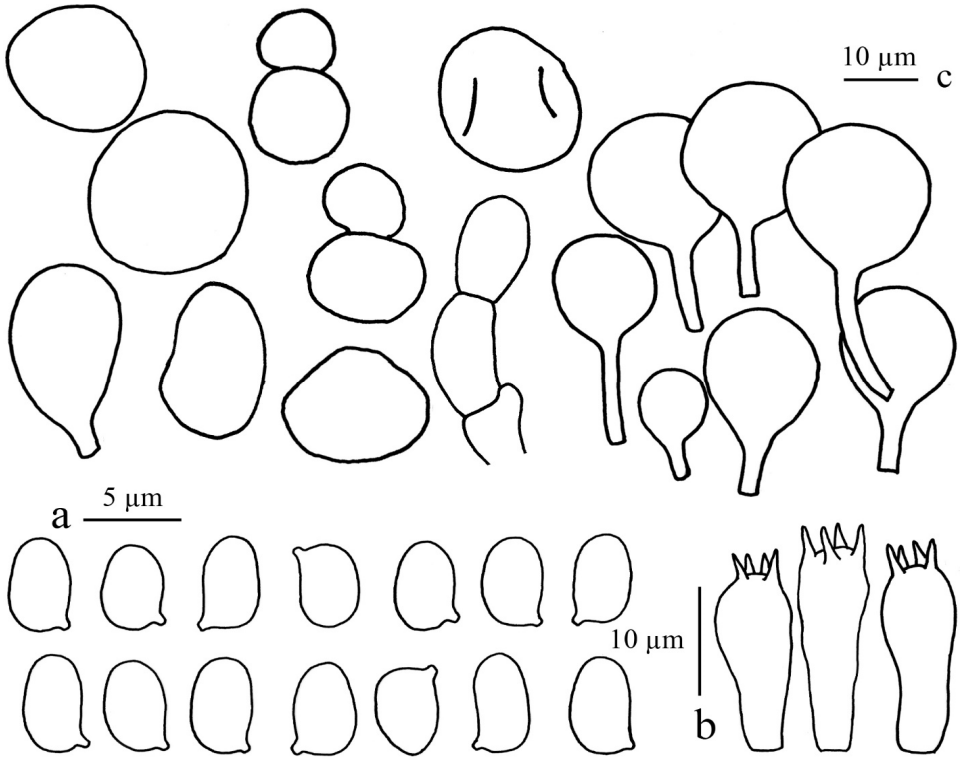

Figure 5. Microcharacters of *Cystolepiota thailandica* (MFLU22-0017, holotype). (**a**) = basidiospores, (**b**) = basidia, (**c**) = pileus covering cells.

Etymology –the species epithet 'thailandica' is derived from the name of the country where the material was collected.

Diagnosis—*Cystolepiota thailandica* has basidiomata that are covered with greyish-orange granules or powder, white lamellae, hyaline and ellipsoid basidiospores, clavate basidia, no cheilocystidia or pleurocystidia, a pileus and stipe covering made up of globose to subglobose and sphaeropedunculate elements and clamp connections.

Holotype—Thailand, Chiang Rai Province, Muang District, Mae Fah Luang University Campus, 11 January 2019, Yuan S. Liu, STO-2019-062 (MFLU22-0017).

Pileus 25–32 mm diam., first hemispherical to convex, expanding to convex or slightly plano-concave, with inflexed margin, when young granulose to powdery, greyish orange (6B3–5), soon breaking up, granulose to powdery at center toward margin, sometimes fragile when mature, on white powdery background; margin covered with granulose to powdery velar remnants, concolorous to those on surface. Lamellae free, 1.5–2 mm wide, crowded, white, broadly ventricose, with 3–4 lamellulae, with concolorous eroded edge, turning light brown to brown (7D5–8) when touched. Stipe 25–35 × 1.5–2.5 mm, cylindrical, completely covered with white to greyish orange (6B3–5) granulose to powdery velar remnants, on white background, turning light brown to brown (7D5-8) when touched. Annulus an annular zone with white to greyish-orange (6B3–5) remnants. Context white in pileus, 1–1.5 mm thick at center; hollow in stipe and concolorous with surface. Odor and taste not observed. Spore print white.

Basidiospores (50,2,2) 4.0–5.0 × 1.8–2.5 µm, avl × avw = 4.30 × 2.24 µm, Q = 1.7–2.0, avQ = 1.91, ellipsoid in frontal view, ellipsoid in side-view, slightly thick-walled, smooth, hyaline, non-dextrinoid, non-amyloid. Basidia 15–20 × 5.0–7.0 µm, short clavate to clavate, thin-walled, hyaline, four-spored. Cheilocystidia absent. Pleurocystidia absent. Pileus covering an epithelium composed of globose to subglobose and sphaeropedunculate element cells, 10–35 µm wide, hyaline to pale brown parietal pigments. Stipe covering an epithelium similar to that on pileus. Clamp connections present in all tissues.

Habitat and habit—growing solitary or in a small group with few basidiomata, saprotrophic on humus-rich soil with dead leaves. Found under cultivated trees of *Ficus annulata* Blume.

Additional materials examined—Thailand, Chiang Rai Province, Muang District, Mae Fah Luang University Campus, 11 January 2019, Yuan S. Liu, STO-2019-063 (MFLU22-0018).

Notes—*Cystolepiota thailandica* is not related to any species in the genus by morphology. *Cystolepiota seminuda* (Lasch) Bon is similar to *C. thailandica* by having globose and sphaeropedunculate element cells on pileus and stipe covering and an absence of cheilo- and pleurocystidia but is different in having a white to pale pink or yellowish pileus and stipe covering and slightly larger basidiospores (3.5–5.5 × 2.0–3.0 µm) [29]. *Cystolepiota bucknallii* (Berk. and Broome) Singer & Clémençon is different by having a lilac or violaceous powdery covering on the pileus and stipe, pale yellow to pastel yellow lamellae, and lacking sphaeropedunculate element cells [27,28]. *Cystolepiota pseudogranulosa* (Berk. & Broome) Pegler, described from Sri Lanka, differs in the strongly dextrinoid spores and the irregularly shaped pileus covering elements [31].

The phylogram shows that *C. thailandica* is separated from the other species for which sequence data are available (Figure 1).

3.2.3. *Cystolepiota rhodella* Sysoup. and Thongkl., sp. nov.

MycoBank: MB843146; FoF number: FoF 10599; Figures 6 and 7.

Etymology—the name "rhodella" is from the pinkish-ruby-brown color of the basidiomata.

Diagnosis—*Cystolepiota rhodella* has basidiomata covered with violet-brown to greyish-ruby flocculose squamules, white lamellae with violet-brown edge, oblong- ovoid basidiospores, clavate basidia, no pleurocystidia, abundant moniliform to flexuous cheilocystidia with a long apical excrescence and containing pale yellow mucilaginous contents, pileus and stipe covering epithelioid, made up of globose to subglobose cells and clamp connections.

Holotype—Lao PDR, Oudomxay Province, Xay District, Houay Houm Village, 20°31′11″ N, 101°53′27″ E, alt. 985–940 m, 12 September 2014, P. Sysouphanthong, P1484 (HNL501799).

Pileus 10–32 mm diam., first conical to paraboloid, expanding to campanulate or convex, often umbonate with broad umbo, with straight to incurved margin, completely covered by flocculose squamules when young, violet-brown (10E4–8 and 11E6–8) to greyish ruby or ruby (12C5–8, 12D5–8, and 12E5–8), darker at center, on drying becoming darker, greyish brown to dark brown (8F3–4), on white to pinkish-white or pale red (8A2–3) background; margin with flocculose squamules, concolorous with those on surface, often with white to orange grey (5B2) cortina connecting with stipe when young. Lamellae free, white to whitish, 2–4 mm wide, broadly ventricose, with wavy eroded edge, concolorous with squamules on pileus and stipe, with three lamellulae. Stipe 25–40 × 2–5 mm, cylindrical, covered with crowded flocculose squamules, concolorous with those on pileus, on white to pinkish white or pale red (8A2–3) background, with white rhizomorphs at base; hollow. Annulus an annular zone with concolorous flocculose squamules at upper part of stipe, with concolorous cortina as on pileus margin. Context white in pileus and up to 2 mm thick at umbo, white in stipe. Odor and smell not observed. Spore print white to whitish.

Basidiospores (50,2,2) 3.8–4.2 × 2–3 µm, avl × avw = 4 × 2.4 µm, Q = 1.4–1.9, avQ = 1.7, oblong-ovoid in side view, some with straight base, and with rounded or slightly acute apex, ellipsoid to oblong in frontal view, hyaline, thick-walled, dextrinoid, non-amyloid. Basidia 13–15 × 4.5–6 µm, clavate, hyaline, slightly thick-walled, four-spored. Lamella edge sterile, with abundant cheilocystidia. Cheilocystidia 35–40 × 4–7 µm, moniliform to flexuous with long appendiculate apex, some narrowly lageniform, with pale yellow mucilaginous contents, hyaline and slightly thick-walled. Pleurocystidia absent. Pileus covering an irregular epithelium composed of globose (12–65 µm diam.) to subglobose (17–23 × 12–18 µm) elements in upper layers, with oblong (25–38 × 15–20 µm) elements in lower layers, thin-walled, with pale brown parietal and intracellular pigments. Stipe covering in the squamules an irregular epithelium same as in pileus covering. Clamp connections present.

Habitat and habit—growing solitary or in a small group, saprotrophic on humus-rich soil with dead leaves, in various habitats, e.g., mixed deciduous forest with *Castanopsis* spp. and *Lithocarpus* spp. as dominant tree species in northern Laos, in dipterocarp forest with *Shorea obtusa* Wall. ex Blume dominant, and in deciduous forest of *Ficus annulata* Blume in northern Thailand.

Materials examined: Thailand, Chiang Rai Province, Pa Daed District, Pha Ngae Sub-district, 19°34′57″ N, 100°00′51″ E, alt. 510–540 m, 28 August 2018, P. Sysouphanthong, PS2018-138 (MFLU22-0019); Chiang Mai, Mae Taeng District, Pong Duad Village, 16°06′16.1″ N, 99°43′07.9″ E, alt. 780–805 m, 9 August 2007, P. Sysouphanthong, PNG31 (MFLU09-0050).

Note—*Cystolepiota rhodella* was found in dipterocarp forests (and other habitats) in Northern Laos and Northern Thailand. It is easily recognized by violet-brown or greyish-ruby to ruby squamules on basidiomata and white lamellae with violet-brown edges. Because of its unique color and the colored lamella edge, the species stands out, and there are few similar species. *Melanophyllum haematospermum* (Bull.) Kreisel differs from *C. rhodella* by cinnamon or pale brownish-gray to light grayish-brown basidiomata and cinnamon-red to dark brown lamellae [32]. Microscopically it differs in the subtly ornamented colored spores and cystidia that are in general absent or, if present, inconspicuous; the exsiccata turn black [33]. The phylogram (Figure 1) showed that *C. rhodella* is separated from other species; it is basal to the clade made up of specimens of *C. hetieri* (Boud.) Singer.

Figure 6. *Cystolepiota rhodella*. (**a**–**c**) = MFLU22-0019, (**d**,**e**) = HNL501799 (holotype), (**f**,**g**) = (MFLU09-0050).

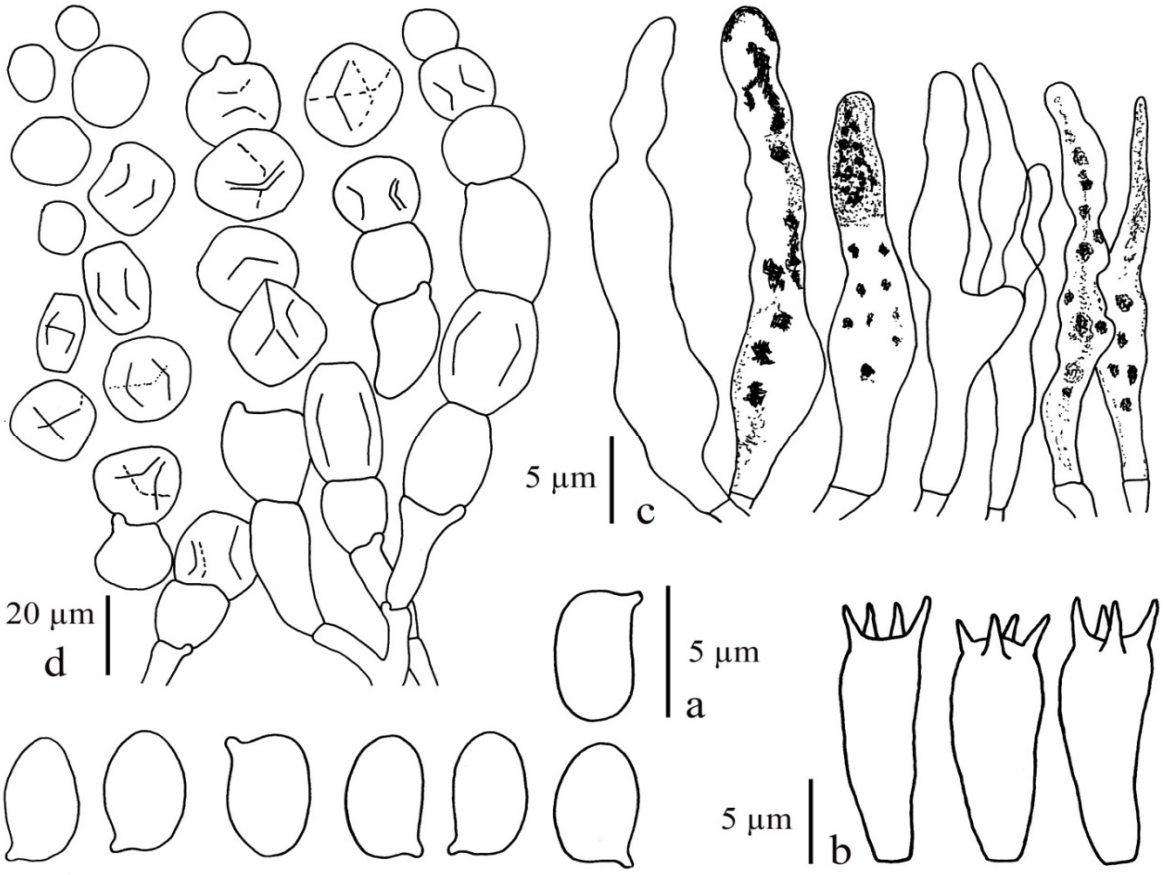

Figure 7. *Cystolepiota rhodella* (HNL503227, holotype) (**a**) = basidiospores, (**b**) = basidia, (**c**) = cheilocystidia, (**d**) = pileus covering elements.

Author Contributions: All authors have contributed equally to this work. Conceptualization, P.S. and E.C.V.; methodology, P.S., N.T. and E.C.V.; software, P.S., Y.S.L. and E.C.V.; validation, E.C.V. and P.S.; formal analysis, N.T., P.S. and E.C.V.; investigation, Y.S.L. and E.C.V.; resources, P.S. and Y.S.L.; data curation, E.C.V. and P.S.; writing—original draft preparation, P.S., E.C.V., N.T. and Y.S.L.; writing—review and editing, E.C.V. and N.T.; visualization, P.S. and N.T.; supervision, E.C.V. and N.T.; project administration, P.S. and N.T.; funding acquisition, N.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Thailand research fund grant "Study of saprobic Agaricales in Thailand to find new industrial mushroom products" (Grant No. DBG6180015), Thailand Science Research and Innovation (TSRI) grant "Macrofungi diversity research from the Lancang-Mekong Watershed and surrounding areas" (Grant No. DBG6280009), and Mae Fah Luang University grant "Taxonomy, phylogeny of micro and macro fungi in Mae Fah Luang University premises" (Grant No. 64316001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data can be found within the manuscript.

Acknowledgments: We would like to thank the Center of Excellence in Fungal Research, Mae Fah Luang University, for providing laboratory facilities for morphological study and depositing the herbarium. The Biotechnology and Ecology Institute, Ministry of Science and Technology of Laos, is also thanked for depositing the herbarium.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. He, M.Q.; Zhao, R.L.; Hyde, K.D.; Begerow, D.; Kemler, M.; Yurkov, A.; McKenzie, E.H.C.; Raspé, O.; Kakishima, M.; SánchezRamírez, S.; et al. Notes, outline and divergence times of Basidiomycota. *Fungal Divers.* **2019**, *99*, 105–367.
2. Kalichman, J.; Kirk, P.M.; Matheny, P.B. A compendium of generic names of agarics and Agaricales. *Taxon* **2020**, *69*, 425–447. [CrossRef]
3. Bon, M. Novitates 4. Famille Lepiotaceae Roze ex Overeen. *Doc. Mycol.* **1993**, *22*, 27–32.
4. Vellinga, E.C.; Noordeloos, M.E. Glossary. In *Flora Agaricina Neerlandica 5*; Noordeloos, M.E., Kuyper, T.W., Vellinga, E.C., Eds.; A.A. Balkema Publishers: Tokyo, Japan, 2001; pp. 6–11.
5. Vellinga, E.C. Genera in the family Agaricaceae—Evidence from nrITS and nrLSU sequences. *Mycol. Res.* **2004**, *108*, 354–377. [CrossRef] [PubMed]
6. Hou, Y.J.; Ge, Z.W. New species of *Echinoderma* and *Lepiota* (Agaricaceae) from China. *Phytotaxa* **2020**, *447*, 221–236. [CrossRef]
7. Donk, M.A. The generic names proposed for *Agaricaceae*. *Bei. Nova Hedwigia* **1962**, *5*, 1–320.
8. Fayod, V. Prodrome d'une histoire naturelle des agaricinés. *Ann. Soc. Nat. Bot. VII* **1889**, *9*, 181–411.
9. Knudsen, H. Notes on *Cystolepiota* Sing. and *Lepiota* S.F. Gray. *Bot. Tidsskr.* **1978**, *73*, 124–136.
10. Bon, M. Les lépiotes de l'herbier Boudier au Muséum National d'Histoire Naturelle de Paris. *Doc. Mycol.* **1977**, *7*, 11–22.
11. Bon, M. Les genres *Echinoderma* (Locq. ex Bon) st. nov. et *Rugosomyces* Raithelhuber ss. lato. *Doc. Mycol.* **1991**, *21*, 61–66.
12. Vellinga, E.C. Phylogeny of *Lepiota* (Agaricaceae)—evidence from nrITS and nrLSU sequences. *Mycol. Prog.* **2003**, *2*, 305–322. [CrossRef]
13. Chandrasrikul, A.; Suwanarit, P.; Sangwanit, U.; Lumyong, S.; Payapanon, A.; Sanoamuang, N.; Pukahuta, C.; Petcharat, V.; Sardsud, U.; Duengkae, K.; et al. *Checklist of Mushrooms (Basidiomycetes) in Thailand*; Office of Natural Resources and Environmental Policy and Planning: Bangkok, Thailand, 2011.
14. Sysouphanthong, P.; Bouamanivong, S.; Salichan, T. *Some mushrooms in Houayyang Preserves*; Biotechnology and Ecology Institute: Vientiane, Laos. (In Lao)
15. Kornerup, A.; Wanscher, J.H. *Methuen Handbook of Colour*, 3rd ed.; Eyre Methuen: London, UK, 1978.
16. White, T.J.; Bruns, T.; Lee, S.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: San Diego, CA, USA, 1990; pp. 315–322.
17. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes—Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **1993**, *2*, 113–118. [CrossRef] [PubMed]
18. Rehner, S.A.; Samuels, G.J. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol. Res.* **1994**, *98*, 625–634. [CrossRef]
19. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef] [PubMed]
20. Liu, Y.J.; Whelen, S.; Hall, B.D. Phylogenetic relationships among Ascomycetes: Evidence from an RNA polymerase II subunit. *Mol. Biol. Evol.* **1999**, *16*, 1799–1808. [CrossRef] [PubMed]
21. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef] [PubMed]
22. Katoh, K.; Toh, H. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* **2008**, *9*, 286–298. [CrossRef]
23. Stamatakis, A.; Hoover, P.; Rougemont, J. A rapid bootstrap algorithm for the RAxML webservers. *Syst. Biol.* **2008**, *75*, 758–771. [CrossRef]
24. Ronquist, F.; Huelsenbeck, J.P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **2003**, *19*, 1572–1574. [CrossRef]
25. Nylander, J.A.A. *Modeltest v2: Program Distributed by the Author*; Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden, 2004.
26. Page, R.D. TreeView: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **1996**, *12*, 357–358.
27. Vellinga, E.C. Lepiotaceous fungi in California, U.S.A. 4. Type studies of *Lepiota fumosifolia* and *L. petasiformis*. *Mycotaxon* **2007**, *98*, 225–232.
28. Vellinga, E.C.; Huijser, H.A. Notes on *Cystolepiota*: Sections *Cystolepiota* and *Pulverolepiota*. *Persoonia* **1998**, *16*, 513–526.
29. Vellinga, E.C. Notes on *Cystolepiota seminuda*. *Persoonia* **1987**, *13*, 321–325.
30. Paraíso, M.; Maurice, J.P.; Normand, A.C.; Fouchier, F.; Roux, P. *Cystolepiota oliveirae* sp. nov., récoltée au Portugal sur tronc de fougère arborescente morte. *Mycol. Montenegrina* **2016**, *19*, 21–31.
31. Pegler, D.N. *Agaric Flora of Sri Lanka*; Kew Bulletin Additional Series XII; HMSO: London, UK, 1986.
32. Seok, S.J.; Jin, Y.J.; Kwon, S.W.; Kim, Y.S.; Kim, W.G. A taxonomic study of genus *Melanophyllum* in Korea. *Korean J. Mycol.* **2013**, *41*, 205–211. [CrossRef]
33. Vellinga, E.C. Lepiota (Pers.: Fr.) S.F. Gray, *Cystolepiota* Sing. and *Melanophyllum* Velen. In *Flora Agaricina Neerlandica*; Noordeloos, M.E., Kuyper, T.W., Vellinga, E.C., Eds.; A.A. Balkema Publishers: Rotterdam, The Netherlands, 2001; Volume 5, pp. 109–151.