

## Article

# Survey of *Volvariella* (Agaricales, Basidiomycota) including Two New Species, *V. neovolvacea* and *V. thailandensis*, from Northern Thailand

Jaturong Kumla<sup>1,2,\*</sup>, Nakarin Suwannarach<sup>1,2</sup> , Nopparat Wannathes<sup>3</sup> and Saisamorn Lumyong<sup>1,2,4</sup>

- <sup>1</sup> Research Center of Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai 50200, Thailand; nakarin.su@cmu.ac.th (N.S.); saisamorn.l@cmu.ac.th (S.L.)  
<sup>2</sup> Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand  
<sup>3</sup> Microbiology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok 65000, Thailand; wnopparat@psru.ac.th  
<sup>4</sup> Academy of Science, The Royal Society of Thailand, Bangkok 10300, Thailand  
\* Correspondence: jaturong.kumla@cmu.ac.th; Tel.: +66-87-192-6527

**Abstract:** During the period from 2018 to 2020, a survey of macrofungi in northern Thailand was conducted and seven specimens of *Volvariella* were collected. The morphological characteristics and phylogenetic analyses of the internal transcribed spacers (ITS) and large subunit (nrLSU) of the nuclear ribosomal DNA (rDNA) sequence data indicated that three specimens belonged to *V. bombycina* and *V. volvacea*. Four other specimens were distinct from all other known species within the genus *Volvariella*. We described these specimens as two new species, namely *V. neovolvacea* and *V. thailandensis*. Comprehensive descriptions, illustrations, line drawings, and a phylogenetic tree are provided to show the position of the two new species. The comparisons between morphologically similar and phylogenetically related species are also discussed.



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**Keywords:** gill fungi; phylogeny; saprophytic fungi; taxonomy; tropical area

## 1. Introduction

The genus *Volvariella* was first proposed in 1898 with *V. argentina* as the species type. *Volvariella* species are known to be widely distributed throughout Africa, Asia, Australasia, Europe, and America [1–8]. They are recognized as saprobes and are mostly found on the ground in grasslands or forests. They have also been found to grow on dead wood and on the litter of the forest floor [7–10]. *Volvariella* has been characterized by pluteoid basidiomata, the presence of volva, a pink to pinkish brown spore print, ovoid to elongated basidiospores with a smooth surface, and by appearing inamyloid [10–12]. Currently, *V. volvacea* is commercially cultivated as an edible mushroom in Asia, especially in China, India, the Philippines, and Thailand [13,14]. *Volvariella* has been placed within the families *Amanitaceae* [15], *Agaricaceae* [16], and *Pluteaceae* [11] of the order *Agaricales*. In 2006, this genus was placed in the family *Pluteaceae* based on phylogenetic analyses [17]. However, a later molecular study conducted by Justo et al. [18] separated the *Volvariella* species into two genera, namely *Volvariella* and *Volvoopluteus*. *Volvoopluteus* was placed in the family *Pluteaceae*; however, *Volvariella* formed a more distant position from the family *Pluteaceae* and was more closely related to genera *Cantharocybe* and *Cuphophyllum* in the family *Hygrophoraceae*. Therefore, *Volvariella* should be treated as *incertae sedis* of the order *Agaricales* [18]. According to the *Dictionary of Fungi* by Kirk et al. [6] and He et al. [19], about 50 species of *Volvariella* have been reported worldwide. However, there are 143 records of *Volvariella* names in the Index Fungorum (Index Fungorum 2021, accessed on February 2022), including specific and infraspecific taxa, illegitimate names, invalid names, and names widely accepted as taxonomic synonyms. These names can include species that have not yet been well-documented and may also include misidentifications.

Only eight species of *Volvariella*, namely *V. bombycina*, *V. cubensis*, *V. hypopithys*, *V. pseudovolvaacea*, *V. pulla*, *V. pusilla*, *V. rostricystidiata*, and *V. volvaacea*, have been previously recorded from Thailand [6,20,21]. Many studies have proposed that Thailand has proven to be a hot spot for novel species discovery [22]. Recently, this has been further evidenced by the discovery of many new species of macrofungi [23–25]. In this study, we found seven *Volvariella* specimens during our investigations of macrofungi in northern Thailand during the period from 2018 to 2020. Amongst these, two new species, *V. neovolvaacea* and *V. thailandensis*, were introduced based on studies of their morphological and molecular data. We used a combined analysis of ITS and nrLSU of the rDNA sequence data to confirm the taxonomic status and infer the phylogenetic position of these two species.

## 2. Materials and Methods

### 2.1. Sample Collection

*Volvariella* was surveyed at Chiang Mai, Mae Hong Son, and Sukhothai provinces, Thailand during the rainy seasons of the years 2018 to 2020. The collection in Sukhothai province was granted permission from the Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand (document No. 0907.4/13696). Basidiomata were collected and wrapped in aluminum foil and kept in plastic boxes. Specimens were dried in a hot air oven at 45 °C until they were completely dried. They were then kept in a plastic zip-locked bag and deposited in the Herbarium of Bangkok Forest Herbarium (BKF), Bangkok, Thailand and Sustainable Development of Biological Resources (SDBR-CMU), Faculty of Science, Chiang Mai University, Thailand. MycoBank numbers are provided [26].

### 2.2. Morphological Observation

Macromorphological data were derived from fresh specimens. Color names and codes followed Kornerup and Wanscher [27]. Micromorphological data were derived from dried specimens that were rehydrated in 95% ethanol, followed by distilled water, 5% KOH, Congo red solution, or Melzer's reagent. Micromorphological characteristics were examined under a light microscope (Nikon Eclipse Ni U, Tokyo, Japan). Size data of the anatomical structure (e.g., basidiospores, basidia, and cystidia) are based on at least 50 measurements of each structure using the Tarosoft (R) Image Frame Work program. For basidiospore statistics, measurements are presented as (a)b–c–d(e), where 'a' and 'e' are the extreme values, 'b–d' is the range containing 90% of all values, and 'c' is the average. Q represents the ratio of length divided by the width of each basidiospore and Q represents the average Q of all specimens  $\pm$  standard deviation.

### 2.3. DNA Extraction, Amplification and Sequencing

Genomic DNA of dry specimens (5 mg) was extracted using a Genomic DNA Extraction Mini-Kit (FAVORGEN, Ping-Tung, Taiwan). The ITS region was amplified by polymerase chain reaction (PCR) using ITS4 and ITS5 primers [28] under the following thermal conditions: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min on a peqSTAR thermal cycler (PEQLAB Ltd., Fareham, UK). The nrLSU region was amplified with LROR and LRO5 primers [29] under the following thermal conditions: 94 °C for 2 min, 30 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min, and 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light. PCR products were purified using a PCR Clean-Up Gel Extraction NucleoSpin<sup>®</sup> Gel and PCR Clean-Up Kit (Macherey–Nagel, Düren, Germany) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sequencing reactions were performed and the sequences were automatically determined in the genetic analyzer at 1st Base Company (Kembangan, Malaysia) using the PCR primers mentioned above.

#### 2.4. Sequence Alignment

Analysis of the sequences was conducted by similarity searches using the BLAST program available at NCBI (<http://blast.ncbi.nlm.nih.gov>, accessed on 21 December 2021). The sequences from this study, previous studies, and the GenBank database (with  $\geq 60\%$  query coverage and  $\geq 85\text{--}100\%$  sequence similarity) were selected and listed in Table 1. Multiple sequence alignment was performed with MUSCLE [30]. The alignments were manually checked. The combined ITS and nrLSU alignment was deposited in TreeBASE under the study ID number 29067.

**Table 1.** Details of sequences used in the molecular phylogenetic analysis.

Taxa	Strain/Voucher	Country	GenBank Accession Number		Reference
			ITS	nrLSU	
<i>Cantharocybe virosa</i>	SDBR-CMUNK0280	Thailand	MG694692	MG694690	[31]
<i>Cuphophyllus yacurensis</i> <sup>T</sup>	QCAM5891	Ecuador	NR166388	KY780119	[32]
<i>Volvariella aethiops</i>	K(M):195631	United Kingdom	MZ159529	–	Unpublished
<i>Volvariella bombycina</i>	AJ244	Spain	HM562212	HM562256	[18]
<i>Volvariella bombycina</i>	SDBR-CMUNK0726	Thailand	OM417508	OM373644	This study
<i>Volvariella bombycina</i>	xj2018071604	China	MT351048	–	Unpublished
<i>Volvariella caesinotincta</i>	MA54717	Spain	HM562211	HM562255	[18]
<i>Volvariella diplasia</i>	CBS 355.64	India	MH858454	MH870086	[21]
<i>Volvariella dunensis</i>	JAC10587	New Zealand	MN738630	MN738630	Unpublished
<i>Volvariella dunensis</i>	SCM3513	Spain	JF415140	–	[33]
<i>Volvariella guttulosa</i> <sup>T</sup>	LE 313326	Vietnam	–	NG068890	[4]
<i>Volvariella hypopithys</i>	TO AV137	Italy	HM246492	HM246488	Unpublished
<i>Volvariella krizii</i> <sup>T</sup>	PR1516257	Czech Republic	–	MK770133	[34]
<i>Volvariella krizii</i>	BRNM 290000	Czech Republic	–	MK770133	[34]
<i>Volvariella lepiotospora</i>	AJ155	USA	HM562214	HM562259	Unpublished
<i>Volvariella lepiotospora</i>	FLAS-F-61505	USA	MH281874	–	Unpublished
<i>Volvariella morozovae</i> <sup>T</sup>	LE 313229	Vietnam	MF377507	MF377508	[32]
<i>Volvariella morozovae</i>	LE 313323	Vietnam	MK882995	–	[32]
<i>Volvariella murinella</i>	GLM:GLM-F42624	Germany	MK412400	MK412400	Unpublished
<i>Volvariella murinella</i>	GLM:GLM-F43392	Germany	MK412361	–	Unpublished
<i>Volvariella neovolvoacea</i> <sup>T</sup>	SDBR-CMUNK0758	Thailand	OM417503	OM373653	This study
<i>Volvariella neovolvoacea</i>	SDBR-CMUNK0760	Thailand	OM417505	OM417507	This study
<i>Volvariella nivea</i> <sup>T</sup>	GDGM25489	China	FJ749127	–	[35]
<i>Volvariella niveosulcata</i> <sup>T</sup>	LE 313329	Vietnam	MK882996	MK883002	[4]
<i>Volvariella mullicystidiata</i>	SP393639	Brazil	EU920671	EU920670	[3]
<i>Volvariella perciliata</i>	SP393635	Brazil	EU920672	EU920668	[3]
<i>Volvariella ptilotricha</i> <sup>T</sup>	LE 313324	Vietnam	NR164612	MK882997	[4]
<i>Volvariella pulla</i> <sup>T</sup>	LE 313325	Vietnam	NR164613	MK883003	[4]
<i>Volvariella pulla</i>	MFLU 19-1534	Thailand	MT074696	–	[6]
<i>Volvariella pusilla</i>	TO AV139	Italy	HM246494	HM246479	Unpublished
<i>Volvariella rava</i> <sup>T</sup>	GDGM41955	China	KP784686	–	[36]
<i>Volvariella reidii</i>	F1-1.1	Finland	MK770139	MK770131	[34]
<i>Volvariella reidii</i>	F1-1.2	Finland	MK770140	MK770132	[34]
<i>Volvariella rostricystidiata</i> <sup>T</sup>	MFLU 19-1528	Thailand	MT074694	–	[6]
<i>Volvariella rostricystidiata</i>	MFLU 19-1531	Thailand	MT074695	–	[6]
<i>Volvariella cf. sathei</i>	Hama193	Niger	KF926663	–	[37]
<i>Volvariella sathei</i>	AMH 9436	India	JN792550	–	[38]
<i>Volvariella strangulata</i>	TO AV141	Italy	HM246493	HM246484	Unpublished
<i>Volvariella surrecta</i>	GLM:GLM-F61563	Germany	MK412358	MK412358	Unpublished
<i>Volvariella surrecta</i>	AJ55	Spain	HM562213	HM562254	[18]
<i>Volvariella taylorii</i>	AJ54	Portugal	HM562210	HM562260	[18]
<i>Volvariella terrea</i>	LUG11010	France	JF415141	–	[33]
<i>Volvariella thailandensis</i> <sup>T</sup>	SDBR-CMUNK0957	Thailand	OM417510	OM417509	This study
<i>Volvariella thailandensis</i>	SDBR-CMUNK0958	Thailand	OM417510	OM386669	This study

Table 1. Cont.

Taxa	Strain/Voucher	Country	GenBank Accession Number		Reference
			ITS	nrLSU	
<i>Volvariella turcica</i> <sup>T</sup>	OKA-TR17.1	Turkey	MK770135	MK770128	[34]
<i>Volvariella turcica</i>	OKA-TR17.2	Turkey	MK770136	MK770129	[34]
<i>Volvariella volvacea</i>	H6	Nigeria	KC894929	–	[39]
<i>Volvariella volvacea</i>	OE-273	India	KC14211	–	Unpublished
<i>Volvariella volvacea</i>	BKF10217	Thailand	MN492647	OM373563	This study
<i>Volvariella volvacea</i>	SDBR-CMUNK505	Thailand	OM417506	OM373623	This study

Superscript “<sup>T</sup>” represents type species. “–” represents the absence of sequence data in GenBank database.

### 2.5. Phylogenetic Analyses

Phylogenetic analysis was carried out based on the combined dataset of ITS and nrLSU. *Cantharocybe virosa* and *Cuphophyllus yacurensis* were used as the outgroup. A phylogenetic tree was constructed under maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was carried out using RAXML-HPC2 version 8.2.10 [40] on the CIPRES web portal [41] under the GTRCAT model with 25 categories and 1000 bootstrap replications [42]. BI analysis was performed with MrBayes v3.2.6 [43]. The best substitution models for ML and BI analyses were estimated by Akaike information criterion (AIC) in jModelTest 2.1.10 [44]. The best substitution models were GTR+I+G for ITS and nrLSU. For the BI analysis, six simultaneous Markov chains were run for one million generations with random initial trees, and every 1000 generations were sampled. The burn-in was set to discard the first 2000 of the trees, and the remaining trees were used to construct the 50% majority-rule consensus phylogram with calculated Bayesian posterior probabilities (PP). Branches with bootstrap support (BS) and PP values greater than or equal to 70% and 0.95, respectively, were considered to be significantly supported [45,46]. The tree topologies were visualized in FigTree v1.4.0 [47].

## 3. Results

### 3.1. Sample Collection and Morphological Observation

A total of seven specimens of *Volvariella* were obtained in this study. Morphologically, the specimen voucher SDBR-CMU505 collected from Chiang Mai province and the voucher BKF10217 collected from Sukhothai province were found to be similar to the morphological descriptions of *V. volvacea*. Notably, SDBR-CMUNK0726 collected from Mae Hong Son province was initially identified as belonging to *V. bombycina*. However, the morphological characteristics of the other four specimen vouchers (SDBR-CMUNK0758, SDBR-CMUNK0760, SDBR-CMUNK0957, and SDBR-CMUNK0958) collected from Chiang Mai province differed from the previously known descriptions of *Volvariella* species. These four specimen vouchers were representative of a potential new species. Therefore, the identification was then further confirmed by the multi-gene phylogenetic analyses of ITS and nrLSU sequences.

### 3.2. Phylogenetic Results

The sequences of seven specimens were deposited in the GenBank database (Table 1). The alignment of a combination of ITS and nrLSU genes contained 1989 characters, including gaps (ITS: 1–1039 and nrLSU: 1040–1989). RAXML analysis of the combined dataset yielded the best scoring tree with a final ML optimization likelihood value of  $-13,839.8771$ . The matrix contained 1030 distinct alignment patterns with 49.73% undetermined characters or gaps. The estimated base frequencies were recorded as follows: A = 0.2255, C = 0.2599, G = 0.2697, T = 0.2448; substitution rates AC = 1.1148, AG = 2.3791, AT = 0.9787, CG = 0.8637, CT = 5.0398, GT = 1.0000. The gamma distribution shape parameter alpha was equal to 0.3033, and the tree length was equal to 4.9110. In addition, the final average standard deviation of the split frequencies at the end of the total MCMC generations was

calculated as 0.00826 through BI analysis. Phylograms of the ML and BI analyses were similar in terms of topology (data not shown). Therefore, the phylogram obtained from the ML analysis was selected and presented in this study. The phylogram was comprised of 50 specimens of *Volvariella* and two specimens of the outgroup (*Cantharocybe virosa* SDBR-CMUNK0280 and *Cuphophyllus yacurensis* QCAM5891) (Figure 1). A phylogram clearly separated our four specimens into two monophyletic clades with high support values (BS = 100% and PP = 1.0). Accordingly, they were clearly distinguished from the previously known species of *Volvariella*. Two specimen vouchers, namely SDBR-CMUNK0758 and SDBR-CMUNK0760 (introduced as *V. thailandensis*), formed a sister clade to *V. sathei* with high support (BS = 100% and PP = 1.0). Moreover, the two specimens in this study, SDBR-CMUNK957 and SDBR-CMUNK95 (described here as *V. neovolvacea*), formed a sister clade to *V. thailandensis* and *V. sathei* with 91% BS and 1.0 PP support values. Additionally, the other obtained specimen vouchers, SDBR-CMUNK505 and SDBR-NW1022, were placed within the same clade of *V. volvacea*, while the specimen voucher SDBR-CMUNK0726 was placed within the clade of *V. bombycina*.

### 3.3. Taxonomic Description of New Species

***Volvariella neovolvacea*** J. Kumla, N. Suwannarach and S. Lumyong, sp. nov. Figure 2. MycoBank: 842646.

Diagnosis: Similar to *V. volvacea* but differing in a wider basidiospores.

Etymology: Name derived from the macromorphological similarity to *V. volvacea*.

Holotype: THAILAND, Chiang Mai province, Chiang Mai University campus, 18°48'6" N 98°57'23" E, elevation 331 m, on soil in grassland, July 2020, J. Kumla and N. Suwannarach, SDBR-CMUNK0758.

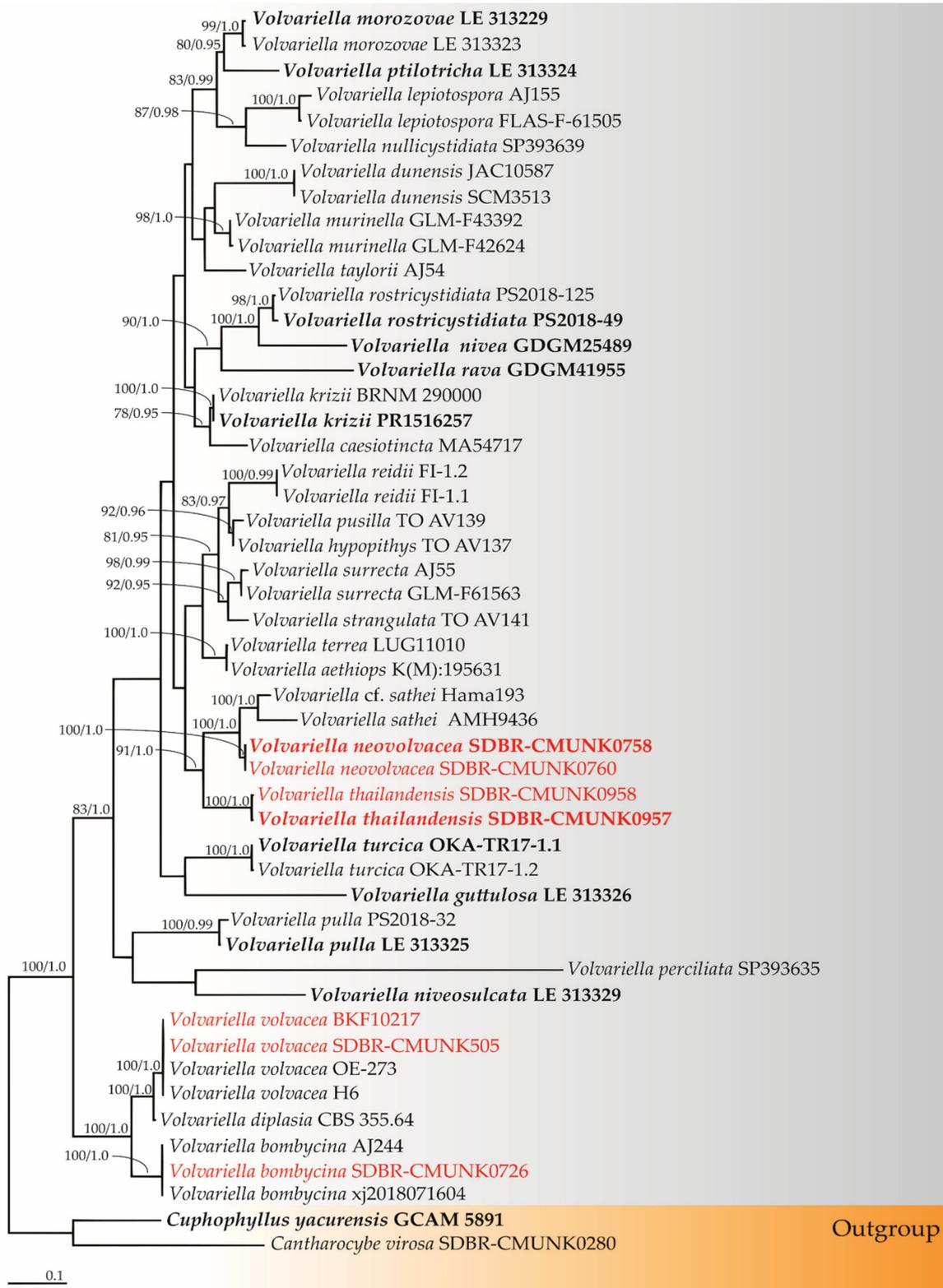
Gene sequences (from holotype): OM417503 (ITS) and OM373653 (nrLSU).

*Pileus* 65–80 mm in diameter, convex, umbo and slightly inflexed margin, brownish orange (5C3) to greyish brown (6E3–7E3), non-hygrophanous, with fibrillose to appressed squamulose surface; margin decurved to straight, thin, crenulate; context soft, white (4A1) to light yellow (4A5). *Lamellae* free, ventricose, close, moderately thin, soft, orange white (6A2) turning pinkish white (7A2) with age; edge finely fimbriate, concolorous with faces; lamellulae present, very variable in length, mostly in one tier. *Stipe* 85–110 × 20–25 mm, central, tapered from the base to apex, solid, grey (6B1), entirely pubescent; context white (4A1) to light yellow (4A5); annulus absent. *Volva* 30–50 mm high, thick, mostly free, saccate, fragile, white (6A1) to greyish brown (6E3). *Spore print* pinkish (7A2).

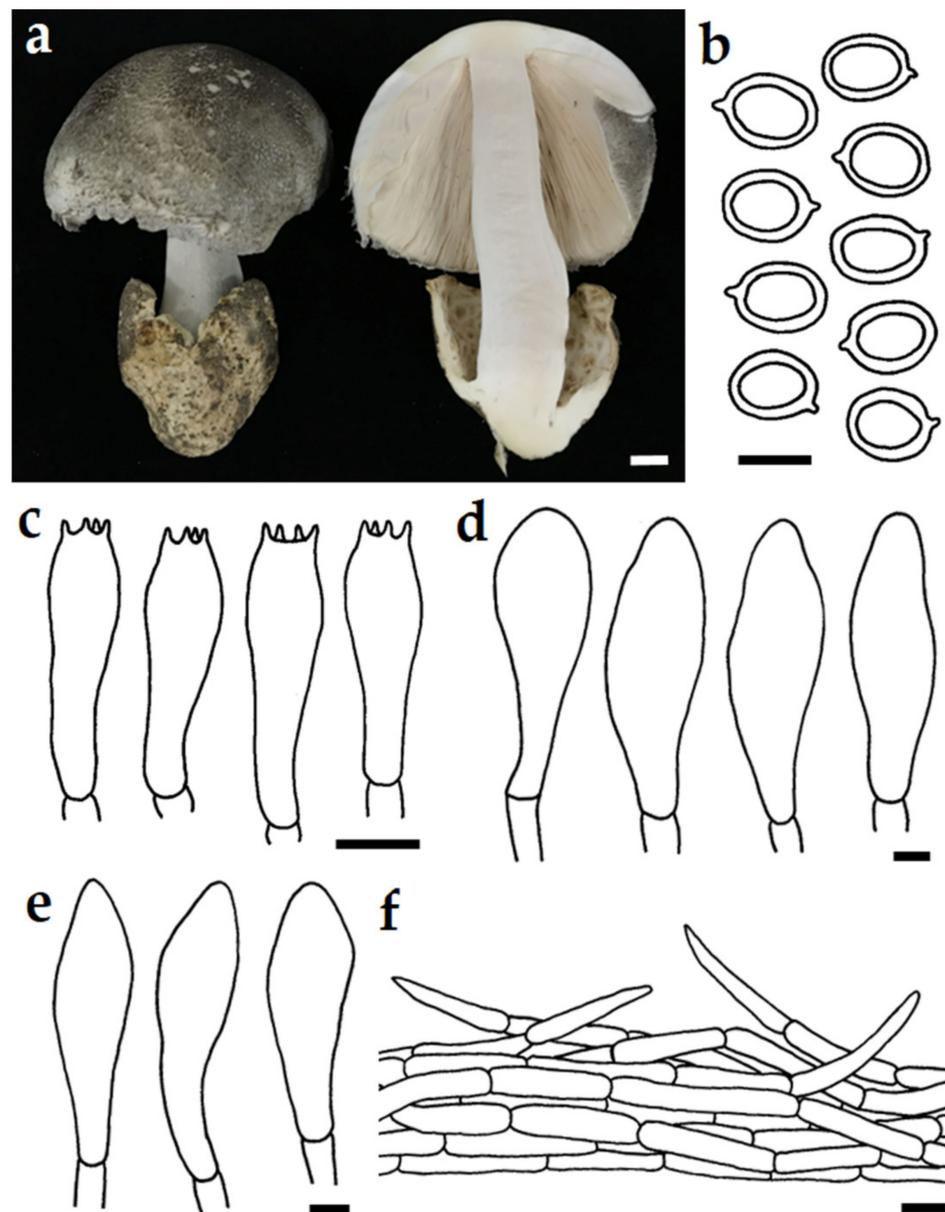
*Basidiospores* (6.5)6.7–7–7.5(8) × (5)5.2–5.5–6(7) μm (n = 50), Q = 1.14–1.45, Q = 1.28 ± 0.1, broadly ellipsoid to ellipsoid, thick-walled, hyaline, inamyloid. *Basidia* 27–40 × 7.5–11 μm, narrowly to broadly clavate, hyaline, 4-spored; sterigmata up to 2.5 μm long. *Pleurocystidia* 45–98 × 23–77 μm, clavate to broadly lageniform, thin-walled, hyaline. *Cheilocystidia* 29–57 × 9–22 μm, clavate to broadly lageniform, thin-walled, hyaline. *Hymenophoral trama* inverse, made up of thin-walled hyaline hyphae, 5–20 μm wide. *Pileipellis* a cutis, with terminal elements 37–98 × 8–16 μm, variable in size, narrowly clavate to clavate, sometimes with a narrowed or slightly strangulated apex, colorless or with pale brown intracellular pigment. *Stipitipellis* a cutis, terminal elements 22–60 × 8–15 μm, cylindrical, sometimes with a narrowed or slightly strangulated apex, colorless or with pale brown intracellular pigment. *Clamp connections* absent in all tissue types.

Ecology and distribution: Fruiting solitary or gregarious on soil in grassland. Known only from northern Thailand.

Additional specimens examined: THAILAND, Chiang Mai province, Chiang Mai University campus, 18°48'7" N 98°57'23" E, elevation 331 m, on soil in grassland, July 2020, N. Suwannarach and J. Kumla, SDBR-CMUNK0760, gene sequences OM417505 (ITS) and OM417507 (nrLSU).



**Figure 1.** Phylogram derived from maximum likelihood analysis of 50 specimens of the combined ITS and nrLSU genes. *Cantharocybe virosa* and *Cuphophyllus yacurensis* were used as the outgroup. The numbers above branches represent bootstrap percentages (left) and Bayesian posterior probabilities (right). Bootstrap values  $\geq 75\%$  and Bayesian posterior probabilities  $\geq 0.90$  are shown. The scale bar represents the expected number of nucleotide substitutions per site. Sequences obtained from this study are in red. Type species are in bold.



**Figure 2.** *Volvariella neovolvacea* SDBR-CMUNK0758 (holotype). Basidiomata (a); Basidiospore (b); Basidia (c); Pleurocystidia (d); Cheilocystidia (e); Pileipellis terminal elements (f). Scale bars: (a) = 10 mm; (b) = 5  $\mu$ m; (c–f) = 10  $\mu$ m.

Note: Morphologically, the size of the pileus (65–80 mm in diameter) in *V. neovolvacea* was clearly larger than those of the brownish orange to greyish brown *Volvariella* species, namely *V. aethiops* (30–50 mm in diameter) [48], *V. caesiotincta* (44 mm in diameter) [49], *V. morozovae* (30–40 mm in diameter) [32], *V. murinella* (30–60 mm in diameter) [50,51], *V. pseudovolvacea* (30–60 mm in diameter) [9], *V. thailandensis* (35–60 mm in diameter), and *V. taylorii* (20–50 mm in diameter) [51] (Table 2). In addition, *V. bakeri*, distributed only in the USA, has a larger pileus size (100 mm in diameter) than *V. neovolvacea* [2,52]. However, the basidiomata size of *V. neovolvacea* was similar to *V. pulla*, *V. rostricystidiata*, *V. terastia*, and *V. volvacea* [4,6,9,51]. The smaller size of the basidiospores in *V. rostricystidiata* and *V. terastia* clearly distinguishes them from *V. neovolvacea* [6,9]. Additionally, the basidiospores of *V. neovolvacea* ( $6.5\text{--}8.0 \times 5.0\text{--}7.0 \mu\text{m}$ ,  $Q = 1.28$ ) were wider than *V. volvacea* ( $6.0\text{--}9.0 \times 4.7\text{--}5.7 \mu\text{m}$ ,  $Q = 1.42$ ) [9,15]. The wider size of pleurocystidia in *V. neovolvacea* clearly differed from *V. pseudovolvacea*, *V. pulla*, *V. rostricystidiata*, and *V. terastia* [4,6].

**Table 2.** Comparison of *V. neovolvacea* and *V. thailandensis* with the closely related species.

<i>Volvariella</i> Species	Distribution	Pileus (mm in Diameter)	Basidiospore (µm)	Basidia (µm)	Pleurocystidia (µm)	Cheilocystidia (µm)	Reference
<i>V. aethiops</i>	France	30–50	7.0–11 × 3.7–7.2	NR	60–100 × 10–25	60–100 × 10–25	[48]
<i>V. bakeri</i>	USA	100	6.9–9.3 × 4.6–6.9	21–35 × 7–11	47–71 × 11–31	27–71 × 11–31	[2,52]
<i>V. caesiointacta</i>	Europe and North Africa	44	6.2–7.7 × 4.2–5.4	21–28 × 6.5–9	35–46 × 10–25	34–65 × 9.6–19	[18,49]
<i>V. morozovae</i>	Vietnam	30–40	5.0–6.2 × 3.3–4.3	13.5–20 × 7–8.5	45–50 × 18–30	40–50 × 13–18	[32]
<i>V. murinella</i>	Europe and Vietnam	30–60	4.8–5.6 × 4.9–6.6	19–25 × 6–7	20–30 × 10–15	45–65 × 8–15	[50,51]
<i>V. neovolvacea</i>	Thailand	65–80	6.5–8.0 × 5.0–7.0	27–40 × 7.5–11	45–98 × 23–77	29–57 × 9–22	This study
<i>V. pseudovolvacea</i>	Sri Lanka	30–60	4.2–5.2 × 2.5–3.5	14–16 × 4.5–6.5	32–45 × 12–16	30–38 × 12–14	[9]
<i>V. pulla</i>	Thailand and Vietnam	40–75	5.5–8.0 × 4.0–5.0	16–19.5 × 7–9	25–56 × 6–32	24–87 × 9–17	[4,6]
<i>V. rava</i>	China	25–60	4.0–6.5 × 4.0–5.0	24–30 × 7–10	35–71 × 11–30	55–80 × 15–25	[36]
<i>V. rostricystidiata</i>	Thailand	55–85	5.0–7.0 × 3.5–5.5	17.5–29 × 7–10	41–117 × 10–25	51–80 × 14–39	[6]
<i>V. taylorii</i>	Europe, South Korea, USA and Vietnam	20–50	5.0–7.0 × 3.0–4.8	20–24 × 6–8	35–49 × 8–10	28–43 × 9–13	[18,51,52]
<i>V. terastia</i>	Sri Lanka	60–110	5.0–6.5 × 4.2–5.2	17–23 × 5.5–7	35–50 × 15–22	32–36 × 10–13	[9]
<i>V. thailandensis</i>	Thailand	35–50	5.5–7.0 × 4.0–6.0	15–35 × 6–8	35–77 × 17–30	32–48 × 6–10	This study
<i>V. volvacea</i>	Asia and East Africa	50–100	6.0–9.0 × 4.7–5.7	30–35 × 7.5–9.0	45–120 × 17–30	40–70 × 10–15	[9,51], This study

“NR” = not reported.

Our phylogenetic results from the combined ITS and nrLSU sequences confirmed that *V. neovolvacea* formed a monophyletic clade, which clearly separated it from the other morphologically related *Volvariella* species (Figure 1). The phylogenetic analysis also supports the determination that *V. neovolvacea* and *V. volvacea* are different species. *Volvariella neovolvacea* forms a sister taxon to *V. sathei*. However, the white to yellowish white pileus of *V. sathei* clearly separates it from *V. neovolvacea* [38]. The ITS sequence of *V. neovolvacea* is 95.64% and 94.00% similar to *V. sathei* (JN792550) and *V. cf. sathei* (KF926663), respectively.

***Volvariella thailandensis*** N. Suwannarach, J. Kumla, and S. Lumyong, sp. nov. Figure 3.

Mycobank: 842647.

Diagnosis: Similar to *V. taylorii* but differing in a wider pleurocystidia and narrower cheilocystidia.

Etymology: Name referred to Thailand, where the new species was found.

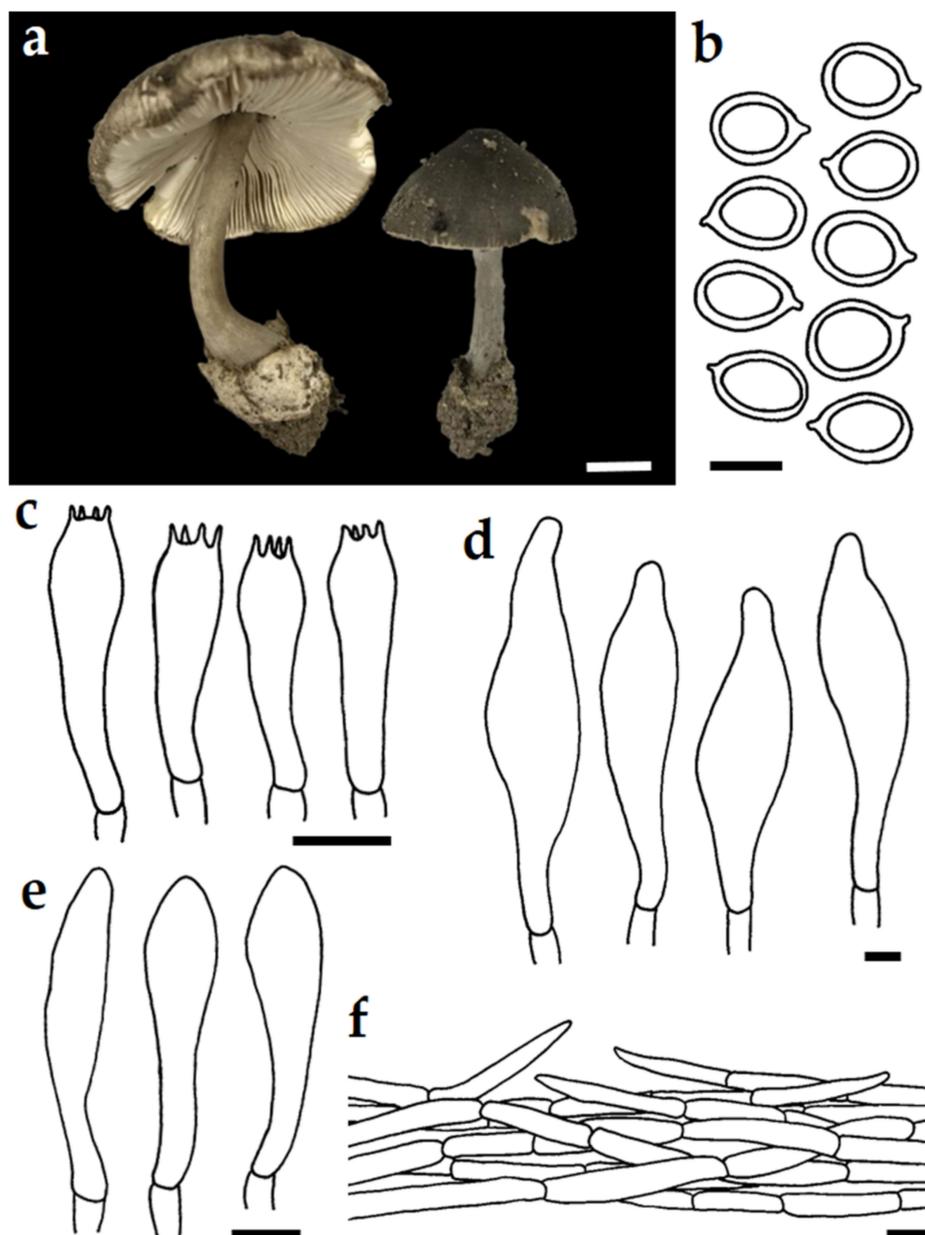
Holotype: THAILAND, Chiang Mai province, Chiang Mai University campus, 18°48'22" N 98°56'51" E, elevation 348 m, on soil in grassland, August 2020, J. Kumla and N. Suwannarach, SDBR-CMUNK0957.

Gene sequences (from holotype): OM417510 (ITS) and OM417509 (nrLSU).

*Pileus* 35–50 mm in diameter, convex to plano-convex with a low broad umbo, greyish brown (6E3–7E3) with slightly darker color at the center, non-hygrophanous, with fibrillose to appressed squamulose surface; margin decurved to straight, thin, crenulate; context soft, light yellow (4A5). *Lamellae* free, ventricose, close, moderately thin, soft, pale orange (6A3) turning brownish orange (6C6) with age; edge finely fimbriate, concolorous with faces; lamellulae present, very variable in length, mostly in one tier. *Stipe* 40–65 × 4–5 mm, central, tapered from the base to apex, solid, greyish brown (6E3) to dark brown (6F5), slightly paler near the base, entirely pubescent; context light yellow (4A5); annulus absent. *Volva* 15–18 mm high, thick, mostly free, saccate, fragile, white (6A1) to greyish brown (6D4). *Spore print* pinkish (7A2).

*Basidiospores* (5.5)6–7–6.5(7) × (4)4.4–5–5.5(6) µm (n = 50), Q = 1.16–1.50, Q = 1.30 ± 0.1, subglobose to broadly ellipsoid, thick-walled, hyaline, inamyloid. *Basidia* 15–35 × 6–8 µm, narrowly to broadly clavate, hyaline, four-spored; sterigmata up to 3 µm long. *Pleurocystidia* 35–77 × 17–30 µm, broadly fusiform to lageniform, thin-walled, hyaline. *Cheilocystidia* 32–48 × 6–10 µm, narrowly to broadly clavate, broadly lageniform, thin-walled, hyaline. *Hymenophoral trama* inverse, made up of thin-walled hyaline hyphae, 3–10 µm wide. *Pileipellis* a cutis, with terminal elements 65–150 × 4–16 µm, variable in size, nar-

rowly clavate to clavate, sometimes with a narrowed or slightly strangulated apex, colorless or with pale brown intracellular pigment. *Stipitipellis* a cutis, terminal elements 35–80 × 6–9 μm, cylindrical, sometimes with a narrowed or slightly strangulated apex, colorless or with pale brown intracellular pigment. *Clamp connections* absent in all tissue types.



**Figure 3.** *Volvariella thailandensis* SDBR-CMUNK0957 (holotype). Basidiomata (a); Basidiospore (b); Basidia (c); Pleurocystidia (d); Cheilocystidia (e); Pileipellis terminal elements (f). Scale bars: (a) = 10 mm; (b) = 5 μm; (c–f) = 10 μm.

Additional specimens examined: THAILAND, Chiang Mai province, Chiang Mai University campus, 18°48′21″ N 98°56′52″ E, elevation 348 m, on soil in grassland, August 2020, J. Kumla and N. Suwannarach, SDBR-CMUNK0958, gene sequences OM417510 (ITS) and OM386669 (nrLSU).

Note: Based on the morphology, the brownish orange to greyish brown pileus and size of *V. thailandensis* were similar to that of *V. aethiops*, *V. caesiointacta*, *V. morozovae*, *V. murinella*, *V. pseudovolvoacea*, *V. rava*, and *V. taylorii* [9,18,32,36,48,50–52] (Table 2). However, the larger size of the basidiospores and basidia in *V. thailandensis* differs from

*V. pseudovolvaacea* [9]. The longer and shorter sizes of basidiospores in *V. thailandensis* clearly distinguishes it from *V. murinella* [50,51] and *V. aethiops* [48], respectively. Additionally, the pleurocystidia ( $35\text{--}77 \times 17\text{--}30 \mu\text{m}$ ) of *V. thailandensis* were found to be wider than of the *V. taylorii* ( $35\text{--}49 \times 8\text{--}10 \mu\text{m}$ ) found in Europe, South Korea, the USA, and Vietnam [18,51,52]. The wider cheilocystidia in *V. caesiointincta* ( $34\text{--}65 \times 9.6\text{--}19 \mu\text{m}$ ) [18,49], *V. morozovae* ( $40\text{--}50 \times 13\text{--}18 \mu\text{m}$ ) [32], *V. pulla* ( $24\text{--}87 \times 9\text{--}17 \mu\text{m}$ ) [4,6], *V. rava* ( $55\text{--}80 \times 15\text{--}25 \mu\text{m}$ ) [36], and *V. taylorii* ( $28\text{--}43 \times 9\text{--}13 \mu\text{m}$ ) [18,51,52] clearly distinguishes them from *V. thailandensis* ( $32\text{--}48 \times 6\text{--}10 \mu\text{m}$ ).

The phylogenetic analyses of the combined ITS and nrLSU sequences confirmed that *V. thailandensis* formed a monophyletic clade, which clearly separated it from the other *Volvariella* species (Figure 1). *Volvariella thailandensis* is closely related to *V. neovolvaacea* and *V. sathei*. However, it is morphologically quite different from *V. sathei* which has white to yellowish white pileus [38]. Moreover, *V. thailandensis* is characterized by smaller basidiomata and wider cheilocystidia than *V. neovolvaacea* (Table 2).

#### 4. Discussion

*Volvariella* is widely distributed in both temperate and tropical areas throughout the world [2,5–8,53]. Traditionally, morphological characteristics have been used in the identification of *Volvariella* species [5,7,9,11]. However, identification can be difficult as some species have similar features. Thus, effective identification can be limited by certain morphological characteristics as well as the different environmental conditions that affect those morphological characteristics. Over the last two decades, molecular phylogeny has been an essential tool in the identification of *Volvariella* species [3,4,6,10,17–19,21,32–39]. Thus, the current classification of the genus *Volvariella* has been based on the combined data of the morphological characteristics and molecular data.

In 2011, six *Volvariella* species (*V. bombycina*, *V. cubensis*, *V. hypopithys*, *V. pseudovolvaacea*, *V. pusilla*, and *V. volvaacea*) have been recorded by mycologists in Thailand according to their morphological characteristics. However, in this regard, there is a lack of available molecular data [20]. Molecular data of *V. volvaacea* from Thai specimen has been reported by Vu et al. [21]. In 2021, a new species (*V. rostricystidiata*) and a new record (*V. pulla*) of *Volvariella* were reported from Thailand by Niego et al. [6]. In this study, two new species of *Volvariella* (*V. thailandensis* and *V. neovolvaacea*) and two previously known species (*V. bombycina* and *V. volvaacea*), collected from northern Thailand, were identified based on their morphological characteristics and phylogenetic analyses. Thus, the number of *Volvariella* species recorded in Thailand has risen to a total of ten species, of which six species were confirmed by morphological and molecular evidence. Nevertheless, four *Volvariella* species listed by Chandrasrikul et al. [20] require further confirmation by molecular data. To our knowledge, our discovery is considerably important in terms of stimulating a deeper investigation of macrofungi in Thailand, and will help researchers better understand the distribution and ecology of *Volvariella*.

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OM417510 and OM417513) and nrLSU (OM373563, OM373623, OM373644, OM373653, OM386669, OM417507 and OM417509).

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

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