รายงานวิจัยฉบับสมบูรณ์

โครงการ Domestication and bioactive evaluation of Thai *Hymenopellis*, *Oudemansiella*, *Xerula* and *Volvariella* species (basidiomycetes) (การเพาะและการประเมินหาสารออกฤทธิ์ทางชีวภาพของเห็ดสกุล *Hymenopellis*, *Oudemansiella*, *Xerula* and *Volvariella* (basidiomycetes))

โดย Emeritus Professor Dr. Kevin David Hyde และคณะ

กันยายน พ.ศ. 2562

สัญญาเลขที่ DBG6180033

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Abstract (บทคัดย่อ)

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Abstract

The edible mushroom, *Volvariella* (paddy straw mushroom) is popular and wildly consumed. The species *V. volvacea* is commonly cultivated and wildly consumed in southeast Asia. In Thailand, *Volvariella* is called “Hed Fang” with only eight species reported from this country. Thus, it is necessary to collect Thai species and strains and introduce and commercialize their use. Another group of mushrooms, *Hymenopellis, Oudemansiella* and *Xerula* belong to the family Physalacriaceae (Basidiomycota). Most genera in this family are edible; however, they are not cultivated in Thailand. This study aimed to collect and select wild edible strains of these mushrooms for taxonomic identification and the relationships of these genera were established through morphological and phylogenetic approaches. In addition, cultivation methods and bioactivities with potential pharmaceutical applications of the selected species were investigated.

The project has duration of one year which commenced on September 2018 and ended on September 2019. A total of 127 mushroom samples with 22 *Volvariella* strains including the control (*V. volvacea*) taken from the local market, 37 *Oudemansiella* strains, 67 *Xerula* strains and 1 strain of *Hymenopellis* were collected

from the different areas in Thailand. This study established new records in Thailand for 2 strains of *Volvariella* and possible 1 new species based on the morphological and phylogenetic analyses.

Moreover, optimized conditions (e.g. media and spawn) for the growths of *Volvariella* and *Xerula* strains were determined. Sorghum was used for spawning of both species. Rice straw was used for the cultivation of *Volvariella* in trays, while saw dusts was prepared in bags for the cultivation of *Xerula* strain. Good mycelial and fruiting body production were observed on the *Xerula* strain while limited growth was noted on the *Volvariella* strain. Furthermore, extraction of bioactive compounds was also initiated but further confirmation of the bioactivities should be determined through laboratory analyses.

Keywords : basidiomycota, cultivation, edible mushrooms, optimal conditions, northern Thailand, tropical

Objectives

- To establish the taxonomy and phylogeny of the genera *Hymenopellis, Oudemansiella, Xerula* and *Volvariella* and discover some potential new cultivatable fungi and isolate strains for conservation and cultivation.
- To discover ways and optimal conditions to cultivate selected *Hymenopellis, Oudemansiella, Xerula* and *Volvariella* species, and introduce new wild mushrooms for cultivation in Thailand.
- To screen the potential pharmaceutical properties of selected species of *Hymenopellis, Oudemansiella, Xerula* and *Volvariella*.

Methodology

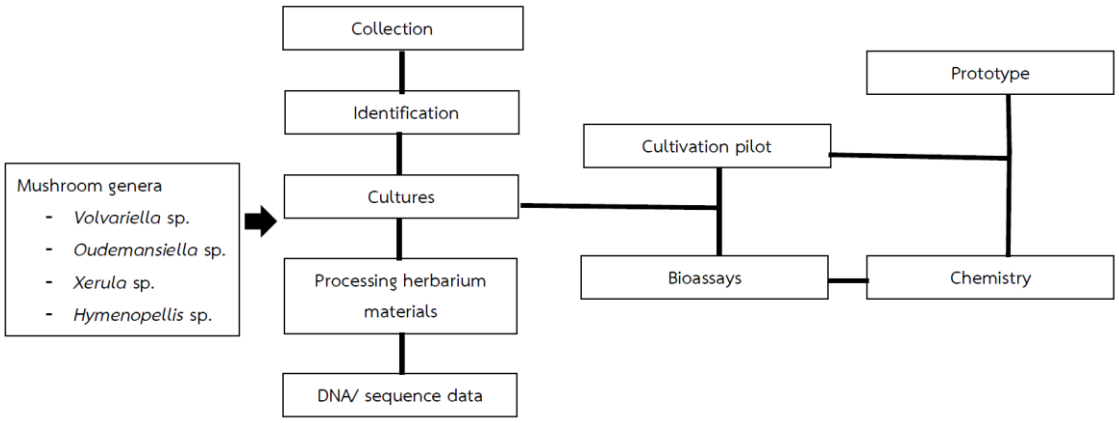

Figure Diagram of work

Mushroom collection

Hymenopellis, Oudemansiella, Xerula and *Volvariella* strains were isolated during the warm wet season. The collection sites in northern Thailand were chosen by altitude and humidity (national park areas were excluded from this study). Cultures of mushrooms obtained were subcultured on agar media for fruiting test and

medicinal screening. Fruiting bodies of mushrooms were collected and used for macro and micro morphological studies and phylogenetic analyses. The cultures and dried fruiting body of mushrooms were deposited in Mae Fah Luang University Culture Collection and Mae Fah Luang University Herbarium.

Identification: Taxonomy and phylogenetic studies

Taxonomy study: mushroom strains were described in detail with macrocharacters and microcharacters from dried fruiting bodies of the mushroom species. The marcromorphological characters include the structure of fruiting bodies, structure of gills and tramas, the chemical and color change and habitat. In addition, micromorphological characters documented pertinent anatomical structures and were carried out under microscope in the laboratory. The size of each microscopic structure was obtained based on at least 20 measurements.

Phylogenetic study: DNA extraction were carried out using CTAB lysis buffer and phenol chloroform as outlined by Doyle & Doyle, 1987; Saghai-Maroof et al., 1984 modified as indicated in Zhao et al., (2011) or using DNA extraction kits. PCR amplification used different genes (e.g. ITS, RPB1, RPB2). The program of PCR was optimized for each gene. The quality and quantity of PCR product were obtained. Products were purified by purification Kit. The sequencing was obtained following the manufacturer's protocols. The sequences were aligned using Clustal X (Thomson et al., 1997) and manually modified in BioEdit. MP, ML or NJ analysis were used for sequence alignment and PAUP*4.0b10 for phylogenetic analysis.This is routine carried out in our laboratory.

Cultivation of selected strains of mushroom

Fruiting test

Testing of cultivability of selected mushrooms was established. The bag cultivation used rubber sawdust as substrate for Physalacriaceae species while the bed cultivation with rice straw was used for *Volvariella* strains. Spawns were inoculated to the bags and bed media. The bags and beds were kept at 25–35 ^{o}C with 70-90% humidity in order to produce fruiting bodies. The experiment was carried out in triplicate.

Develop methods to grow selected mushrooms

Spawn production: cereal grain media and agricultural wasted were used as spawn production substrates. Spawn tubes contained 40-50 gram of each medium and inoculated with mycelial plugs of selected mushrooms. The cultures were incubated at 25–35 ^{o}C. The experiments were carried out in triplicate. The growth rate data of spawn cultures were determined.

Bag cultivation (Physalacriaceae species): The bag cultivation used rubber sawdust and other agricultural waste to cultivate mushrooms. The medium was contained in polypropylene bags with about 800 g of medium then capped with plastic ring and lid. The bags were sterilized at 121°C for 15 minutes or at 90–100°C for 3 h. After the temperature cooled to 25°C, the spawn was inoculated to the bags media. The bags were kept at room temperature with 70-90% humidity in order to produce fruiting bodies. The experiment was

carried out in triplicate. The fruiting bodies, including those with open and closed caps, were manually harvested, counted and weighed daily.

Bed cultivation (*Volvariella* sp.): Agricultural wastes were used as the main substrate for cultivation. The substrates were soaked in water for 12-16 hours and then excess water was removed. Fertilizers and rice bran were added. *Volvariella* spawn were inoculated on the top of the layer. The soaked substrate was covered with a layer of spawn. The trays were covered by polythene sheet and incubated at 30–35 ^{o}C. After full mycelium covered, polythene sheet were removed and moisture was maintained by watering the set-up twice a day. The number of primordia was recorded.

Statistical analysis

The data set was analyzed statistically for variance of means by one-way ANOVA analysis by using Tukey's test. Differences were considered significant for $P < 0.05$.

Screening of bioactive compounds

Small scale fermentation and extraction

Selected strains were cultivated by small scale fermentation in different liquid media. The cultures were harvested after the onset of the stationary phase and organic extracts were prepared following established protocols (Thongbai et al. 2013). In addition, ethanol and water were used for extraction. The extracts may be used in cosmetics and functional foods.

Fruiting body extraction

Fruiting bodies of selected strains which successfully grew were studied. Basidiocarp (0.5 g) was extracted by methanol and ethyl acetate. In addition, ethanol and water were used for extraction as the extract may be used in cosmetics and functional foods. The crude product was dissolved in methanol and kept for further analysis.

Screening of antibacterial, antifungal and antioxidant activity

The bioassays briefly mentioned below are used on a regular basis in one of the Co-Pis (Dr. Charoensup) laboratories. Antibacterial activity of selected extracts will be obtained by disc diffusion method against test microorganisms. Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*), Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), filamentous fungi and yeasts (Ex. *Mucor hiemalis, Candida albicans*) will be used for this study. Crude extracts will be prepared at 20 mg/mL concentration in HPLC grade methanol. A 20 µL of each crude extract will be dropped on sterilized paper discs prior bioassays. Nutrient Agar (NA) petri dishes will be inoculated with 1000 µL of bacterial suspension. The prepared extract discs will be placed on NA agar petri dishes (the bacterial mixed with methanol as a negative control and amoxicillin as a positive control). The inoculated petri dishes will be incubated at 37˚C for 24 hrs (with 3 replicates). The minimum inhibitory concentration (MIC) values will be determined.

Antioxidant activity will be assayed following the method of Brand-William et al. (1995). The crude extracts (5 to 50 mg/mL) will be used in this study. The mixture of each sample will include 30 μL of the crude extract and 220 μL of methanolic solution of DPPH and performed in 96-well microtiter plates. The mixture will be incubated in the darkness at room temperature and measured absorbance at 517 nm every 30 minutes for 2 hrs (by 3 replicates). Butylated hydroxytoluene (BHT) will be used as a standard antioxidant. The DPPH radical scavenging activity percentage was calculated using the following formula.

Scavenging effect (%) = ([Ablank - Asample]/Ablank) x 100
Where,
Ablank = Absorbance of the control solution, DPPH solution without the tested sample.
Asample = Absorbance of the test extract, DPPH solution with the tested sample.

Results

A. Field Collection

Mushroom specimens were collected during the rainy seasons from September 2018-August 2019 from the different locations in northern Thailand namely from Mae Fah Luang University, Huaymaesak, Viengxiengrung District, Phu Chi Fah, Thirng Didtrict, Doi Maesalong, Huai Kang Pla Waterfall Forest Park in Chiangrai province and Mushroom Research Center, Meaon and Me Tang in Chiangmai, Thailand. Moreover, other samples were collected from Kanchanaburi and Phayao provinces, Thailand. There was a total of 127 mushroom samples collected with 22 *Volvariella* strains including the control (*V. volvacea*) taken from the local market, 37 *Oudemansiella* strains, 67 *Xerula* strains and 1 strain of *Hymenopellis* (Table 1). All samples were properly handled and taken to the MFU, CEFR, laboratory to process.

Table 1 List of the mushroom specimens and their collection's location.

No.	Specimen	Code	Location Collected
1	*Volvariella* sp.	MFU-M015	Mae Fah Luang University, Chiang Rai
2	*Volvariella* sp.	PS2018-49	Mae Fah Luang University, Chiang Rai
3	*Volvariella* sp.	PS2018-98	Mae Fah Luang University, Chiang Rai
4	*Volvariella* sp.	PS2018-114	Huaymaesak, Viengxiengrung District, Chiang Rai
5	*Volvariella* sp..	PS2018-125	Mae Fah Luang District, Chiang Rai
6	*Volvariella* sp.	PS2018-69	Mae Fah Luang University, Chiang Rai
7	*Volvariella* sp.	PS2018-42	Mae Fah Luang University, Chiang Rai
8	*Volvariella* sp.	PS2018-32	Mae Fah Luang University, Chiang Rai
9	*Volvariella* sp.	K14-109	MFU, Chiang Rai
10	*Volvariella* sp.	STO-2018-184	Mae Fah Luang University, Chiang Rai
11	*Volvariella* sp.	JP-021	Mae Fah Luang University, Chiang Rai
12	*Volvariella* sp.	SAM06	Temple Near MRC, Chiang Mai

No.	Specimen	Code	Location Collected
13	*Volvariella* sp.	SAM14	MRC, Chiang Mai
14	*Volvariella* sp.	SAM21	Mae Taeng, Chiang Mai
15	*Volvariella* sp.	SAM22	Mae Taeng, Chiang Mai
16	*Volvariella* sp.	SAM31	MRC, Chiang Mai
17	*Volvariella* sp.	LD-137	Bandu, Muang, Chiang Rai
18	*Volvariella* sp.	AGNHMS2019-01	Huai Mae Suk, Chiangrai, Thailand
19	*Volvariella* sp.	AGNHMS2019-02	Huai Mae Suk, Chiangrai, Thailand
20	*Volvariella* sp.	AGNHMS2019-03	Huai Mae Suk, Chiangrai, Thailand
21	*Volvariella* sp.	AGNHMS2019-05	Huai Mae Suk, Chiangrai, Thailand
22	*Volvariella volvacea*	Control	Market place, Chiang Rai
23	*Xerula pudense*	PS2018-123	Doi Maesalong, Mae Fah Luang, Chiang rai, Thailand
24	*Xerula sinopudens*	L-056	Mae Taeng, Chiang Mai
25	*Xerula* sp	PS2018-19	Phu Chi Fah, Thirng Didtrict, Chiang rai, Thailand
26	*Xerula* sp.	PS2018-19	Thoeng District, Chiang Rai
27	*Xerula* sp.	PS2018-123	Mae Fah Luang District, Chiang Rai
28	*Xerula* sp.	PS2018-18	Thoeng District, Chiang Rai
29	*Xerula* sp.	K14-131	MRC, Chiang Mai
30	*Xerula* sp.	K14-132	MRC, Chiang Mai
31	*Xerula* sp.	K14-155	MRC, Chiang Mai
32	*Xerula* sp.	JP-050	Mae Fah Luang District, Chiang Rai
33	*Xerula* sp.	JP-055	Hui Hua Na, Pa Daet, Mae Suai, Chiang Rai
34	*Xerula* sp.	JP-057	Mae Kone, Chiang Rai
35	*Xerula* sp.	L-045	MRC, Chiang Mai
36	*Xerula* sp.	L-046	Temple Near MRC, Chiang Mai
37	*Xerula* sp.	L-047	MRC, Chiang Mai
38	*Xerula* sp.	L-049	Muang, Chiang Mai
39	*Xerula* sp.	L-050	Muang, Chiang Mai
40	*Xerula* sp.	W-316	Temple Near MRC, Chiang Mai
41	*Xerula* sp.	KW-217	Mae Fah Luang District, Chiang Rai
42	Xerula sp.	MRC-7B	MRC, Chiang Mai
43	*Xerula* sp.	MRC-10B	Doi Pha Deang temple, Chiang Mai
44	*Xerula* sp.	MRC-34B	Mae Taeng, Chiang Mai
45	*Xerula* sp.	BZN-35	Chiang Mai
46	*Xerula* sp.	BZN-062	3 km down the road from Tharnthong Lodges, Chiang Rai
47	*Xerula* sp.	BZN-063	3 km down the road from Tharnthong Lodges, Chiang Rai

No.	Specimen	Code	Location Collected
48	*Xerula* sp.	BZN-064	3 km down the road from Tharnthong Lodges, Chiang Rai
49	*Xerula* sp.	AGN2019-01	MFU, Chiangrai
50	*Xerula* sp.	AGNMRC2019-02	Mushroom Research Center, Chiangmai, Thailand
51	*Xerula* sp.	AGNMSC2019-01	Doi Maesalong, Chiang rai, Thailand
52	*Xerula* sp.	AGNMSC2019-02	Doi Maesalong, Chiang rai, Thailand
53	*Xerula* sp.	AGNMSC2019-03	Doi Maesalong, Chiang rai, Thailand
54	*Xerula* sp.	AGNMSC2019-04	Doi Maesalong, Chiang rai, Thailand
55	*Xerula* sp.	AGNMSC2019-05	Doi Maesalong, Chiang rai, Thailand
56	*Xerula* sp.	AGNMSC2019-06	Doi Maesalong, Chiang rai, Thailand
57	*Xerula* sp.	AGNMSC2019-07	Doi Maesalong, Chiang rai, Thailand
58	*Xerula* sp.	AGNMSC2019-08	Doi Maesalong, Chiang rai, Thailand
59	*Xerula* sp.	AGNMSC2019-09	Doi Maesalong, Chiang rai, Thailand
60	*Xerula* sp.	AGNMSC2019-10	Doi Maesalong, Chiang rai, Thailand
61	*Xerula* sp.	AGNMSC2019-11	Doi Maesalong, Chiang rai, Thailand
62	*Xerula* sp.	AGNMSC2019-12	Doi Maesalong, Chiang rai, Thailand
63	*Xerula* sp.	AGNMSC2019-13	Doi Maesalong, Chiang rai, Thailand
64	*Xerula* sp.	AGNMSC2019-14	Doi Maesalong, Chiang rai, Thailand
65	*Xerula* sp.	AGNMSC2019-15	Doi Maesalong, Chiang rai, Thailand
66	*Xerula* sp.	AGNMSC2019-16	Doi Maesalong, Chiang rai, Thailand
67	*Xerula* sp.	AGNMSC2019-17	Doi Maesalong, Chiang rai, Thailand
68	*Xerula* sp.	AGNMSC2019-18	Doi Maesalong, Chiang rai, Thailand
69	*Xerula* sp.	AGNMSC2019-19	Doi Maesalong, Chiang rai, Thailand
70	*Xerula* sp.	AGNMSC2019-20	Doi Maesalong, Chiang rai, Thailand
71	*Xerula* sp.	AGNMSC2019-21	Doi Maesalong, Chiang rai, Thailand
72	*Xerula* sp.	AGNMSC2019-22	Doi Maesalong, Chiang rai, Thailand
73	*Xerula* sp.	AGNMSC2019-23	Doi Maesalong, Chiang rai, Thailand
74	*Xerula* sp.	AGNMSC2019-24	Doi Maesalong, Chiang rai, Thailand
75	*Xerula* sp.	AGNMSC2019-25	Doi Maesalong, Chiang rai, Thailand
76	*Xerula* sp.	AGNMSC2019-26	Doi Maesalong, Chiang rai, Thailand
77	*Xerula* sp.	AGNMSC2019-27	Doi Maesalong, Chiang rai, Thailand
78	*Xerula* sp.	AGNMSC2019-28	Doi Maesalong, Chiang rai, Thailand
79	*Xerula* sp.	AGNMSC2019-29	Doi Maesalong, Chiang rai, Thailand
80	*Xerula* sp.	DMSL-DG004	Doi Maesalong, Chiang rai, Thailand
81	*Xerula* sp.	ML-MO04	Doi Maesalong, Chiang rai, Thailand
82	*Xerula* sp.	MT039	Meaon, Chiangmai
83	*Xerula* sp.	MT033	Meaon, Chiangmai
84	*Xerula* sp.	M2019-002	Meaon, Chiangmai

No.	Specimen	Code	Location Collected
85	*Xerula* sp.	Thai20190070	Huai Kang Pla Waterfall Forest Park, Chiang Rai
86	*Xerula* sp.	AGNBP2019-01	Ban Padeng, Mae Tang, Chiang Mai
87	*Xerula* sp.	AGNBP2019-02	Ban Padeng, Mae Tang, Chiang Mai
88	*Xerula* sp.	AGNBP2019-07	Ban Padeng, Mae Tang, Chiang Mai
89	*Xerula* sp.	AGNBP2019-09	Ban Padeng, Mae Tang, Chiang Mai
90	*Hymenopellis* sp.	STO-2018-001	Thoeng District, Chiang Rai
91	*Oudemanseilla* sp.	BZN-057	3 km down the road from Tharnthong Lodges, Chiang Rai
92	*Oudemansiella* aff. *crassifolia*	L-055	Mae Taeng, Chiang Mai
93	*Oudemansiella canarii*	L-053	Mae Kone, Chiang Rai
94	*Oudemansiella* sp.	DMS14-02	MRC, Chiang Mai
95	*Oudemansiella* sp.	DMS14-22	Temple near MRC, Chiang Mai
96	*Oudemansiella* sp.	DMS14-56	Temple near MRC, Chiang Mai
97	*Oudemansiella* sp.	DMS14-57	Temple near MRC, Chiang Mai
98	*Oudemansiella* sp.	DMS14-140	MRC, Chiang Mai
99	*Oudemansiella* sp.	DMS14-208	MRC, Chiang Mai
100	*Oudemansiella* sp.	DMS14-213	MRC, Chiang Mai
101	*Oudemansiella* sp.	K15-31	Kanchanaburi
102	*Oudemansiella* sp.	K14-171	MRC, Chiang Mai
103	*Oudemansiella* sp.	STO-2018-064	Ban Mung Noen Maprang, Chiang Rai
104	*Oudemansiella* sp.	STO-2018-140	Mae Fah Luang University, Chiang Rai
105	*Oudemansiella* sp.	L-042	Mae Fah Luang District, Chiang Rai
106	*Oudemansiella* sp.	L-044	MRC, Chiang Mai
107	*Oudemansiella* sp.	L-048	Near MRC, Chiang Mai
108	*Oudemansiella* sp.	L-051	Temple Near MRC, Chiang Mai
109	*Oudemansiella* sp.	L-052	Mae Kone, Chiang Rai
110	*Oudemansiella* sp.	L-054	Hui Hua Na, Pa Daet, Mae Suai, Chiang Rai
111	*Oudemansiella* sp.	L-057	MRC, Chiang Mai
112	*Oudemansiella* sp.	L-058	MRC, Chiang Mai
113	*Oudemansiella* sp.	L-059	Mae Kone, Chiang Rai
114	*Oudemansiella* sp.	L-060	Mae Yao, Muang, Chiang Rai
115	*Oudemansiella* sp.	W-093	Mae Taeng, Chiang Mai
116	*Oudemansiella* sp.	BZ-56	Kupanglang Temple, Chun, Phayao
117	*Oudemansiella* sp.	BZ-99	Temple Near MRC, Chiang Mai
118	*Oudemansiella* sp.	LE-027	Chiang Mai
119	*Oudemansiella* sp.	AGNMRC2019-01	Mushroom Research Center, Chiangmai, Thailand
120	*Oudemansiella* sp.	AGNMRC2019-03	Mushroom Research Center, Chiangmai, Thailand

No.	Specimen	Code	Location Collected
121	*Oudemansiella* sp.	MT060	Huai Mae Suk, Chiangrai, Thailand
122	*Oudemansiella* sp.	DMS14-162	MRC, Chiang Mai
123	*Oudemansiella* sp.	AGNBP2019-03	Ban Padeng, Mae Tang, Chiang Mai
124	*Oudemansiella* sp.	AGNBP2019-04	Ban Padeng, Mae Tang, Chiang Mai
125	*Oudemansiella* sp.	AGNBP2019-05	Ban Padeng, Mae Tang, Chiang Mai
126	*Oudemansiella* sp.	AGNBP2019-06	Ban Padeng, Mae Tang, Chiang Mai
127	*Oudemansiella* sp.	AGNBP2019-08	Ban Padeng, Mae Tang, Chiang Mai

B.1 Taxonomy

Photographs of each sample were taken in the field. The fresh samples were described based on the macroscopic characteristics of the basidiocarps especially the prominent structures such as the pileus, lamella, stipe. All samples were dried using the dryer in the lab and were deposited in the MFU herbarium. Tissue culturing of rehydrated gills and spore germination using serial dilution method were also performed to obtain mycelial growth. Microscopic characteristics of each sample were observed under the microscope (e.g. basidiospore, basidium, cystidia) and pictures with calibrations were acquired. The following macro and micro morphological are some of the fungi that have been examined. Macroscopic and microscopic pictures were used to prepare for the photoplates with descriptions (Figure 1-24).

Description of some of the fungi that have been examined

1. ***Volvariella nivea*** (MFU-M015) New Record

Morphological characteristics

Gilled mushroom. *Pileus surface color* white. *Context color* light yellow to white. *Pileus shape* broadly convex. *Pileal surface texture* not striated, velutinous. *Pileus margin* translucent-striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.1. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* light yellow to white. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil.

Basidiospores 5.0–6.6–7.0 × 4.04–4.4–5.5 µm, ellipsoid. *Basidia* 22.3–26.5 × 7.7–10.2 µm, clavate. *Pileipellis* cylindrical, segmented,13.0-14.0 µm diameter. *Cheilocystidia* 35.0–171.6 x 8.08–40.5 µm, broadly clavate with cylindrical peduncule. *Pleurocystidia* present

Figure 1. Basidiocarp of *Volvariella nivea* (MFU-M015).

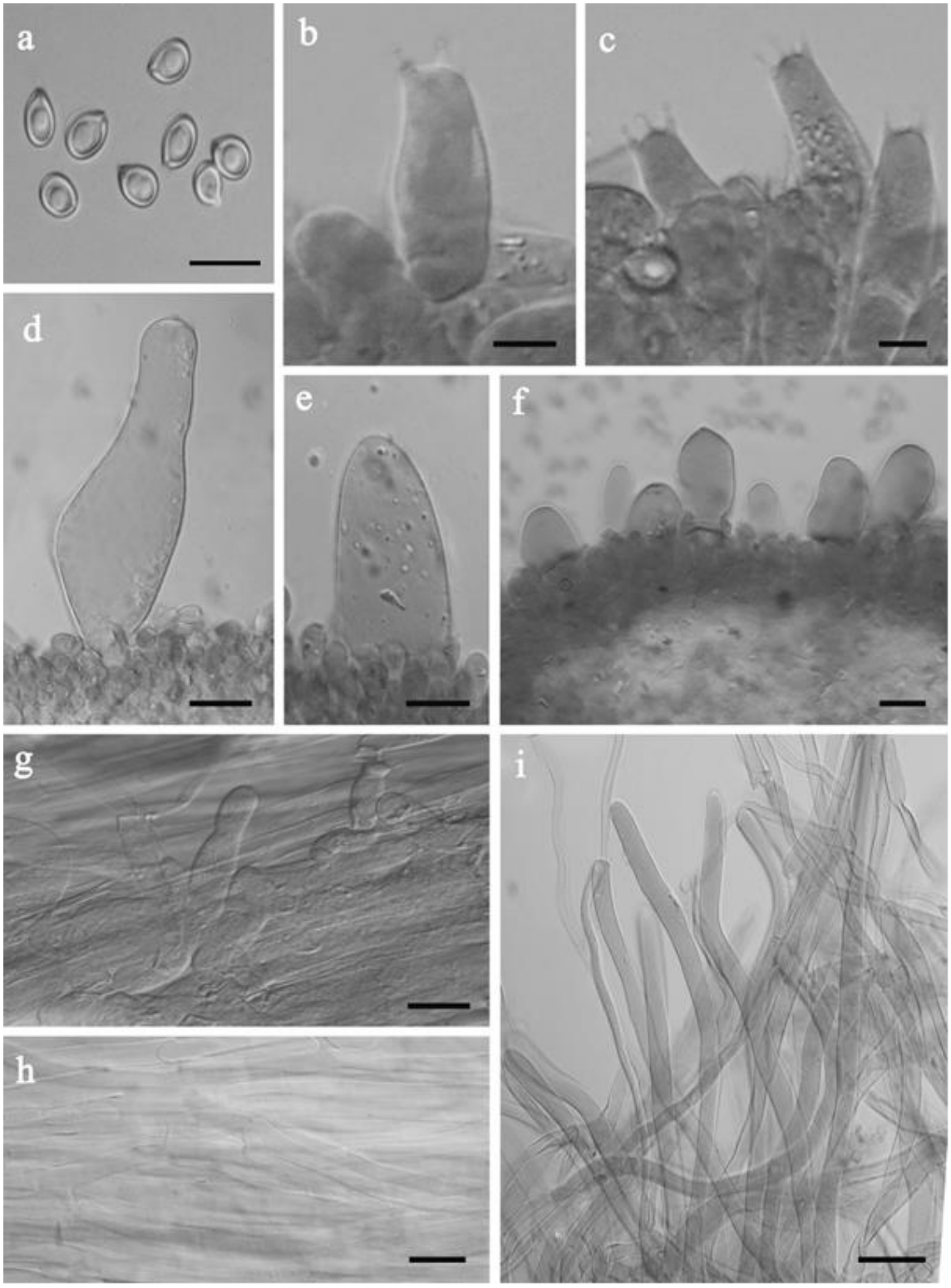

Figure 2. *Volvariella nivea* (MFU-M015). a Basidiospores. b-c Basidium. d-f Cheilocystidia. g-h Stipitipellis. i Pileipellis. Scale bars a= 10 µm, b-c= 5 µm, d-i= 20 µm

2. ***Volvariella pulla*** (PS201832) New Record

Morphological characteristics

Gilled mushroom. *Pileus surface color* dark brown. *Context color* light yellow. *Pileus shape* broadly convex. *Pileal surface texture* vetulinous. *Pileus margin* plicate-striate/ decurved to recurved. Lamellae attachment free. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.1. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* dark brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil

Basidiospores 6.0–6.5–7.5 × 4.06–4.5–4.84 µm, ellipsoid. *Basidia* 15.68–19.44 × 7.06–8.96 µm, clavate. *Pileipellis* cylindrical, segmented,13.0-14.0 µm diameter. *Cheilocystidia* 24.0–86.59 x 8.85–16.75 µm, broadly clavate with cylindrical peduncule. *Pleurocystidia* present

Figure 3. Basidiocarp of *Volvariella pulla* (PS201832)

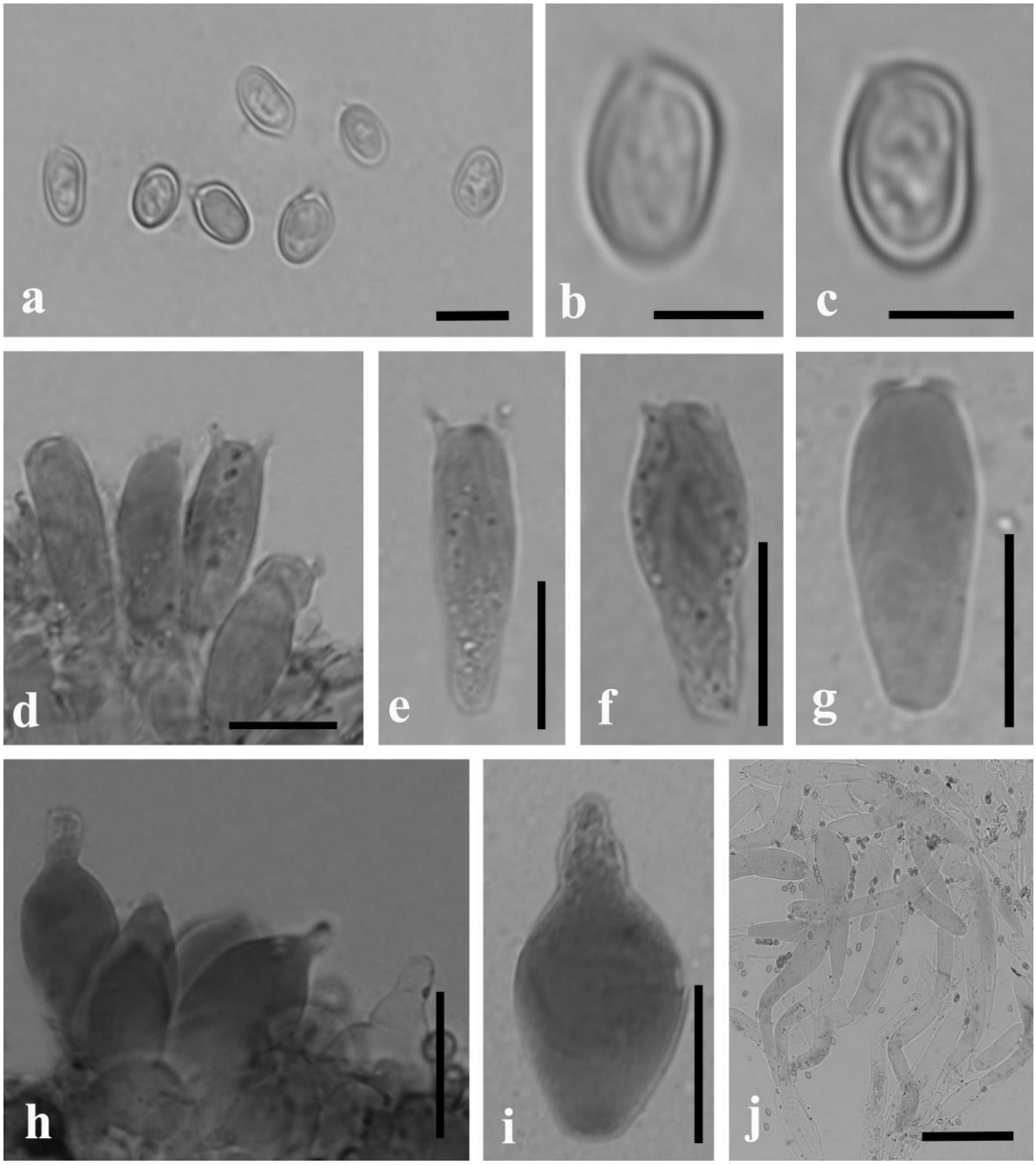

Figure 4. *Volvariella pulla* (MFU-M015). a-cBasidiospores. d-g Basidia. h-i Cheilocystidia. j Pileipellis. Scale bars a-c= 5 µm, d-g= 10 µm, h-i= 20 µm, j=50 µm

3. ***Volvariella* sp. 1** (PS201849, PS201842, PS2018125) New species

Morphological characteristics

Gilled mushroom. Pileus surface color white. Context color light yellow to white. Pileus shape broadly convex. Pileal surface texture not striated, velutinous. Pileus margin translucent-striate/ decurved to recurved. Lamellae attachment free. Lamellae margin even. Lamellae color light brown. Lamellulae orientation regular, no.1. Stipe attachment central. Stipe shape equal. Stipe color light yellow to white. Stipe texture strigose. Annulus absent. Volva present. Odor slightly pungent.

Substrate soil.

Basidiospores 5.0–5.6–6.0 × 4.0–4.2–5.1 µm, round to ellipsoid, 4-spored. Basidia 17.5–22 × 7.5–8.0 µm, clavate. Pileipellis, cylindrical, pigmented with dark brown to light brown, segmented, 16.0-21.2 µm diameter. Cheilocystidia 39.2 x 10.57 µm, broadly clavate with cylindrical peduncule. Pleurocystidia present

Figure 5. Basidiocarp of *Volvariella* sp. 1 (PS201849), collection 1.

Figure 6. Basidiocarp of *Volvariella* sp. 1(PS201842), collection 2

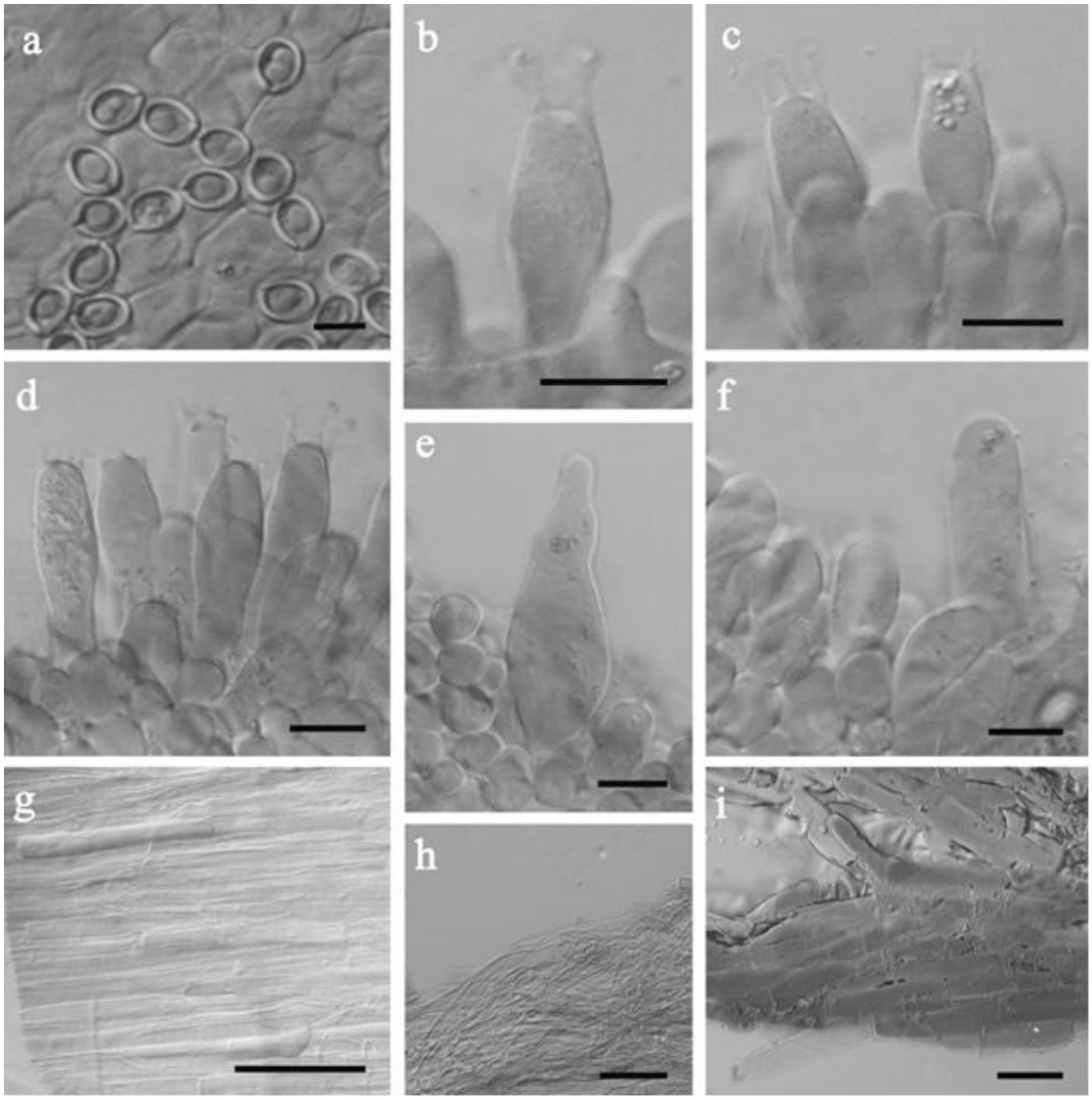

Figure 7. *Volvariella* sp1 (New species). a Basidiospores. b-d Basidia. e-f Cheilocystidia. g-h Stipitipellis. i Pileipellis. Scale bars a= 5 µm, b-f= 10 µm, g-i= 50 µm

4. *Volvariella* sp. (PS201898)

Morphological characteristics

Gilled mushroom. *Pileus surface color* dark brown at the middle becoming light brown towards the margin. *Context color* light yellow. *Pileus shape* broadly convex. *Pileal surface texture* vetulinous. *Pileus margin* plicate-striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.1. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* light brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil.

Figure 8. Basidiocarp of *Volvariella* sp. (PS201898).

5. ***Volvariella* sp.** (PS2018114)

Morphological characteristics

Gilled mushroom. *Pileus surface color* dark brown at the middle becoming light brown towards the margin. *Context color* light yellow. *Pileus shape* broadly convex. *Pileal surface texture* velutinous. *Pileus margin* plicate-striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.1. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* light brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil.

Figure 9. Basidiocarp of *Volvariella* sp. (PS2018114).

6. ***Volvariella* sp.** (PS201869)

Morphological characteristics

Gilled mushroom. *Pileus surface color* dark brown at the middle becoming light brown towards the margin. *Context color* light yellow. *Pileus shape* broadly convex. *Pileal surface texture* vetulinous. *Pileus margin* plicate-striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.1. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* light brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil.

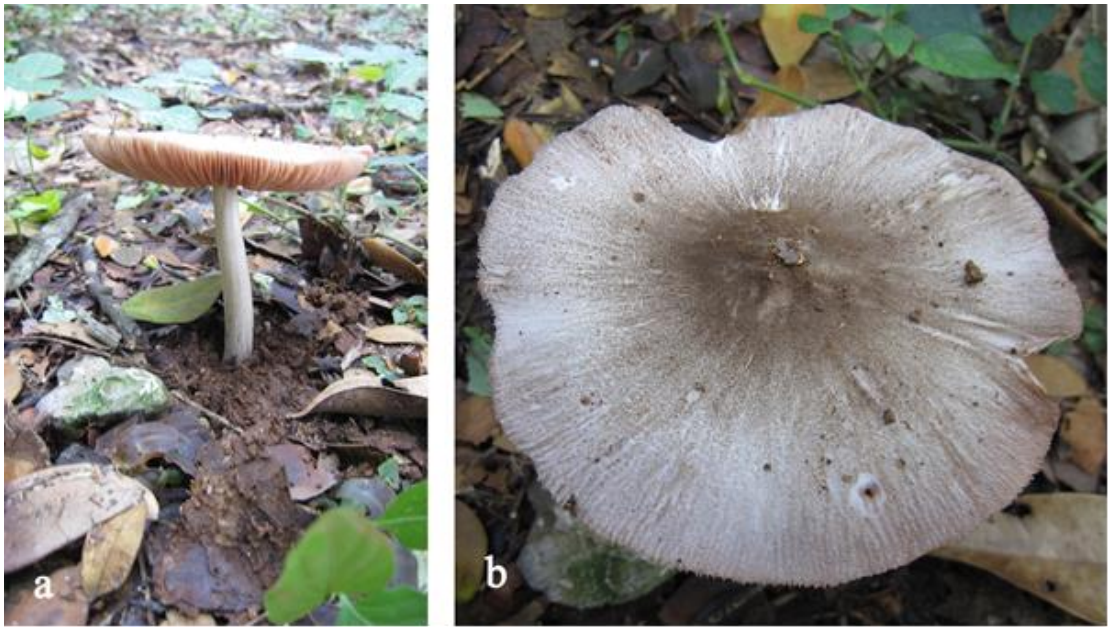

Figure 10. Basidiocarp of *Volvariella* sp. (PS201869)

7. ***Xerula* sp.1** (PS201819)

Morphological characteristics

Gilled mushroom. Pileus surface color dark brown. Context color white. Pileus shape flat. Pileal surface texture slimy. Pileus margin not striate/ decurved to recurved. Lamellae attachment free. Lamellae margin even. Lamellae color white. Lamellulae orientation regular, no.2. Stipe attachment central. Stipe shape equal. Stipe color brown. Stipe texture strigose. Annulus absent. Volva present.

Substrate soil.

Basidiospores 10.0–11.8–12.8 × 9.8–10.5–12.2 µm, round to slightly ellipsoidal. *Basidia* 41.4–50.6× 11.6–13.9 µm, clavate. *Pileipellis* cylindrical, 8.4–10.9 µm diameter. *Cheilocystidia* 62.9–99.8 x 12.7–21.5 µm, broadly clavate with cylindrical peduncule. *Pleurocystidia* present.

Figure 11. Basidiocarp of *Xerula* sp1. (PS201819)

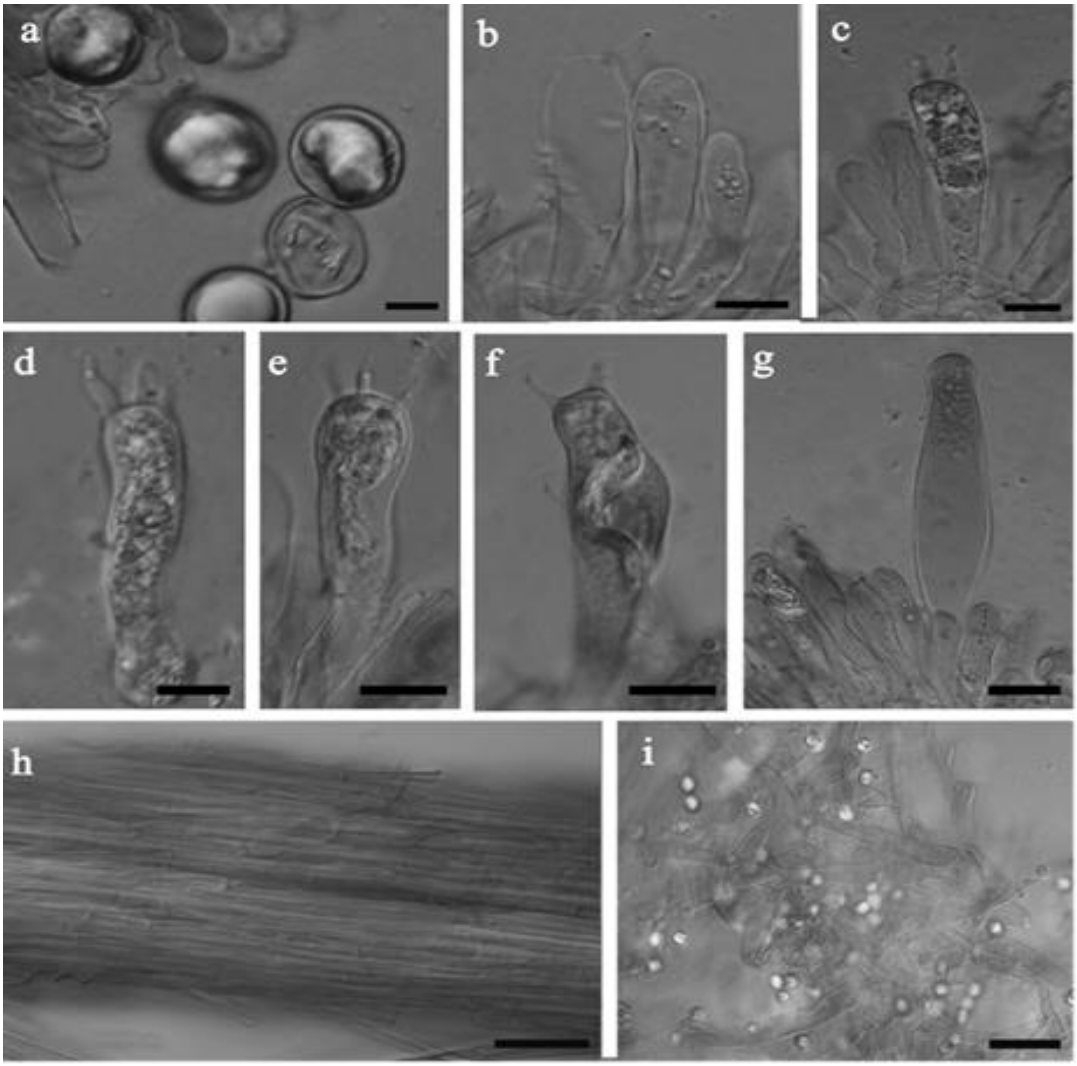

Figure 12. *Xerula* sp. 1 (PS201819). a Basidiospores. b-f Basidia. g Cheilocystidia. h Stipitipellis. i Pileipellis. Scale bars a= 5 µm, b-f= 10 µm, g= 20 µm h-i= 50 µm

8. ***Xerula* sp.** (PS2018123)

Morphological characteristics

Gilled mushroom. *Pileus surface color* dark brown. *Context color* light white. *Pileus shape* broadly convex to flat. *Pileal surface texture* velutinous. *Pileus margin* not striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present.

Substrate trunk.

Basidiospores 8.3–11.5–12.2 × 8.0–11.0–11.5 µm, round to slightly ellipsoid. *Basidia* 41.3–48.9 × 9.8–11.7 µm, clavate. *Pileipellis* cylindrical with trichomes. *Cheilocystidia* 85.0-98.3 x 20.7–25.9 µm, broadly clavate with cylindrical peduncule. *Pleurocystidia* present.

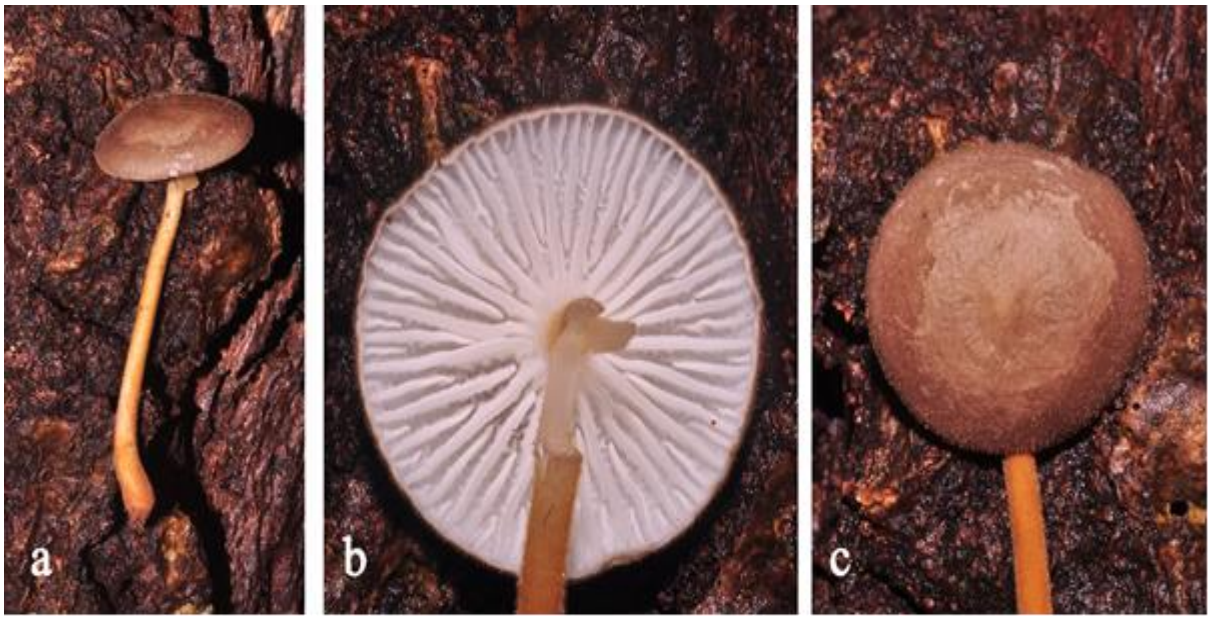

Figure 13. Basidiocarp of *Xerula* sp. (PS2018123)

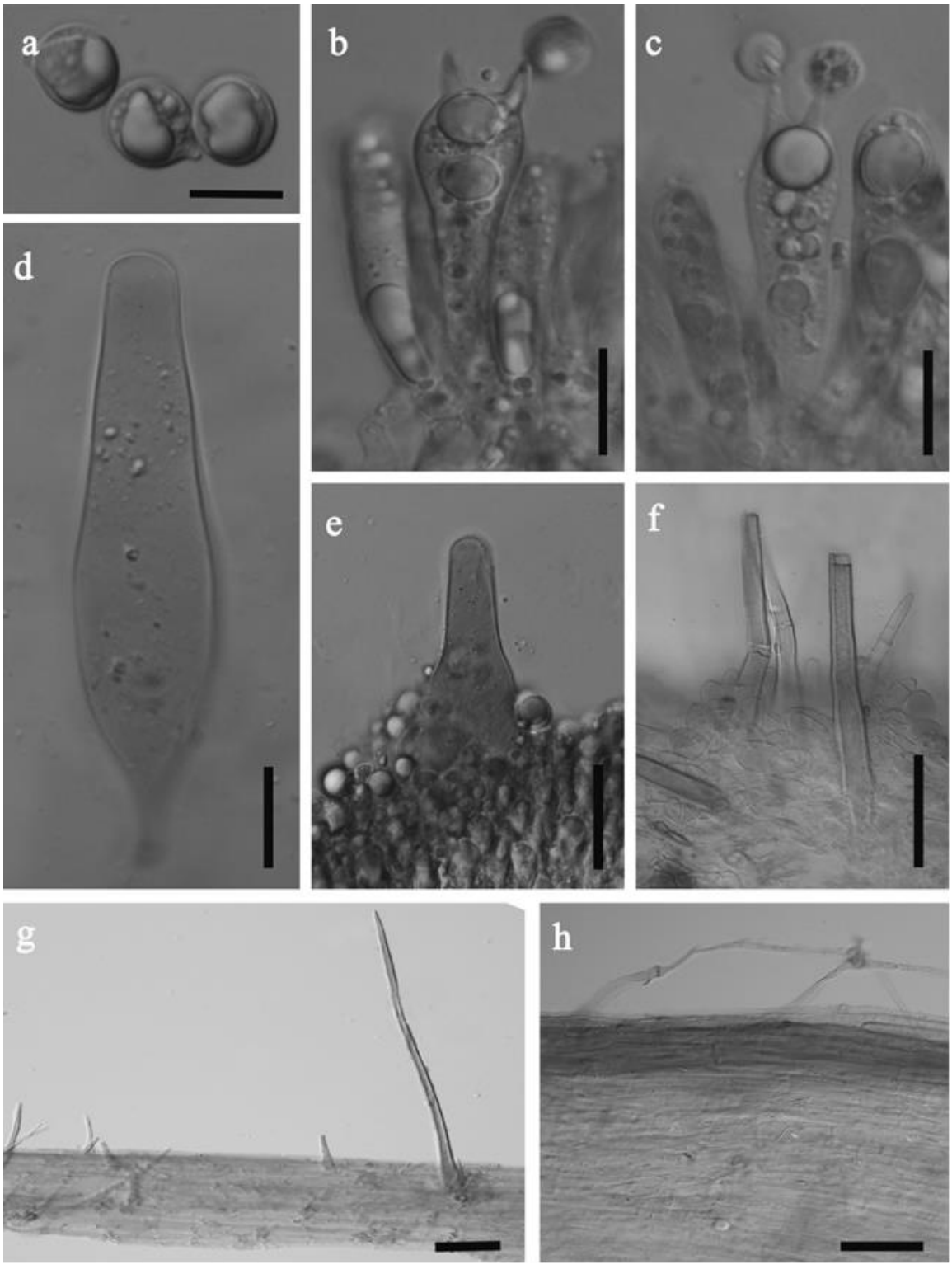

Figure 14. *Xerula* sp. 2 (PS2018123). a Basidiospores. b-c Basidia. d-e Cheilocystidia. f-g Pileipellis. h Stipitipellis. Scale bars a= 10 µm, b-d= 15 µm, e-f= 25 µm g-h= 50 µm

9. *Xerula* sp. (PS201818)

Morphological characteristics

Gilled mushroom. *Pileus surface color* brown in the middle becoming lighter as it goes to the margin. *Context color* light yellow. *Pileus shape* broadly convex. *Pileal surface texture* radially fibrillose. *Pileus margin* plicate-striate/ decurved to recurved. *Lamellae attachment* free. *Lamellae margin* even. *Lamellae color* yellow. *Lamellulae orientation* regular, no.2. *Stipe attachment* central. *Stipe shape* equal. *Stipe color* brown. *Stipe texture* strigose. *Annulus* absent. *Volva* present. *Odor* slightly pungent.

Substrate soil.

Figure 15. Basidiocarp of *Xerula* sp. 3 (PS201818)

10. *Xerula* sp. (AGNBP2019-01)

Morphological characteristics

Gilled mushroom. Substrate soil. Odor slightly pungent. Pileus size 2.8 cm diam. Pileus surface color brown, lighter in the middle. Pileus shape broadly convex, plane with slight umbo. Pileal surface texture slightly villose, with scattered hairs denser towards the margin, radially veinous, rubber-liked. Pileus margin translucent striate. Pileal context color light brown. Lamellae attachment adnexed. Lamellae margin even. Lamellae color white. Lamellulae orientation regular, no.2. Stipe color dark brown. Stipe size 6.8 cm length, 0.4 cm width diam. Stipe attachment central. Stipe shape almost equal but somewhat bigger at the apex. Stipe surface texture strigose. Stipe context light brown, stuffed but a narrow canal in the middle. Stipe base with attached pseudorrhiza. Annulus absent. Volva absent.

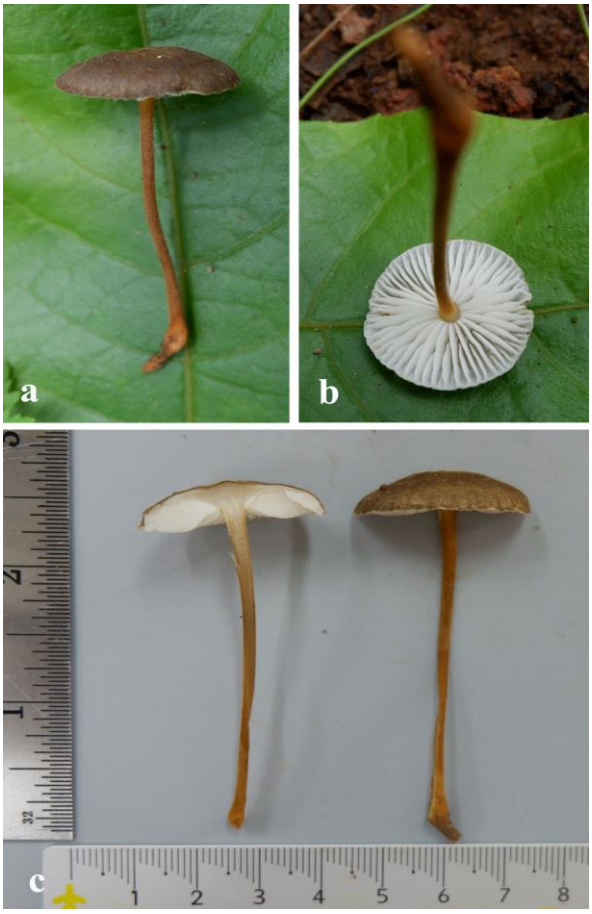

Figure 16. AGNBP2019-01 (*Xerula* sp.) a. basidiome b. Lamellae orientation c. Lamellae attachment with stuffed stipe.

11. *Xerula* sp. (AGNBP2019-02)

Morphological characteristics

Xerula sp. (AGNBP2019-02). Gilled mushroom. *Substrate* soil. *Odor* not determined. *Pileus size* 2.5 cm diam. *Pileus surface color* light brown, slightly darker in the middle. *Pileus shape* plane with slight umbo. *Pileal surface texture* slightly veinous, soft, easily decayed. *Pileus margin* translucent striate. *Pileal context* color light brown. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white to light brown. *Lamellulae orientation* crisped, no.2. *Stipe color* yellowing brown to brown, lighter towards the apex. *Stipe size* 3.5 cm length, 0.4 cm width diam. *Stipe attachment* central. *Stipe shape* almost equal but somewhat bigger at the apex and towards the base. *Stipe surface texture* strigose. *Stipe context* light brown, hollow. *Stipe base* with attached pseudorrhiza. *Annulus* absent. *Volva* absent.

Figure 17. AGNBP2019-02 (*Xerula* sp.) a. basidiome b. Lamellae orientation c. Stipe with pseudorrhiza d. Lamellae attachment with hollow stipe.

12. *Xerula* sp. (AGNBP2019-07)

Morphological characteristics

Xerula sp. (AGNBP2019-07). Gilled mushroom. *Substrate* soil. *Odor* slightly pungent. *Pileus size* 1.5 cm diam. *Pileus surface color* brown, slightly darker in the middle. *Pileus shape* mammilate/ papillate. *Pileal surface texture* radially veinous, rubber-liked. *Pileus margin* translucent striate. *Pileal context color* white to light brown. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe color* dark brown. *Stipe attachment* central. *Stipe shape* tapered at the base. *Stipe surface texture* fibrillose. *Stipe context* light brown, stuffed. *Stipe base* with attached pseudorrhiza. *Annulus* absent. *Volva* absent.

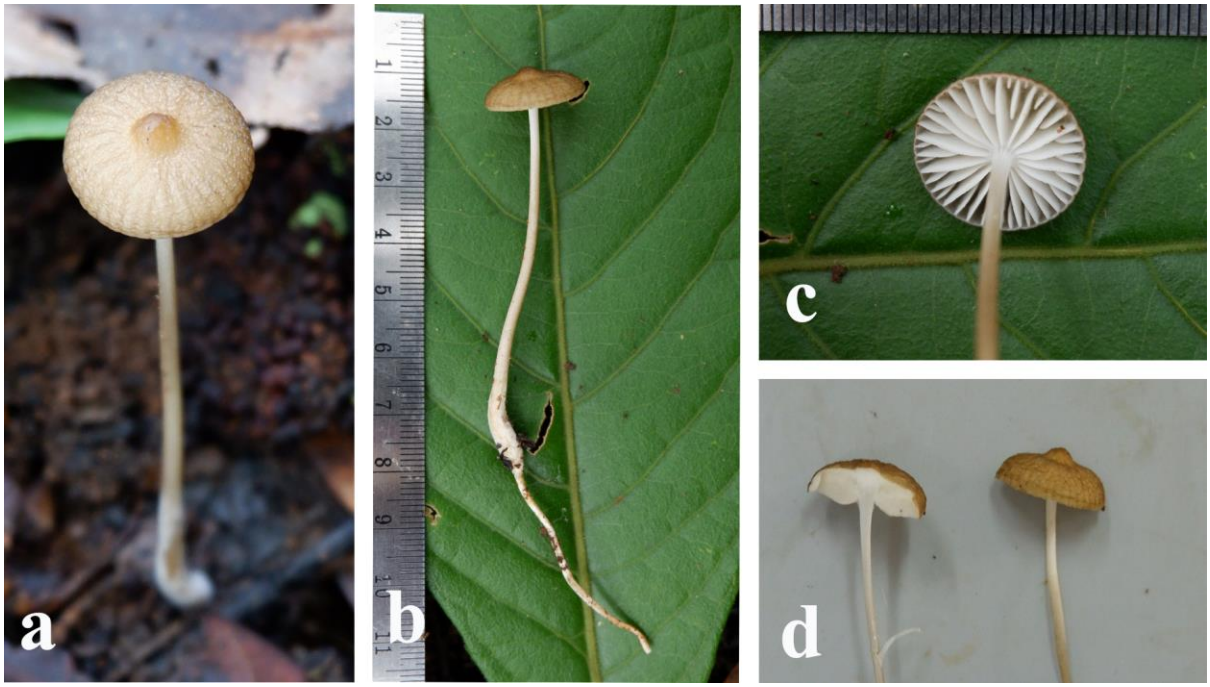

Figure 18. AGNBP2019-07 (*Xerula* sp.) a. basidiome b. basidiome with pseudorrhiza c. lamellae orientation d. Lamellae attachment with stuffed stipe.

13. *Xerula* sp. (AGNBP2019-09)

Morphological characteristics

Xerula sp. (AGNBP2019-09). Gilled mushroom. *Substrate* soil. *Odor* slightly pungent. *Pileus size* 2.5 cm diam. *Pileus surface color* dark brown. *Pileus shape* plane with slight umbo. *Pileal surface texture* slightly radially veinous, rubber-liked. *Pileus margin* translucent striate. *Pileal context* color white to light brown. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe color* brown but lighter towards the apex. *Stipe attachment* central. *Stipe shape* almost equal but somewhat bigger at the apex. *Stipe surface texture* strigose. *Stipe context* white to light brown, stuffed. *Stipe base* with attached pseudorrhiza. *Annulus* absent. *Volva* absent.

Figure 19. AGNBP2019-09 (*Xerula* sp.) a. basidiome b. Lamellae orientation c. lamellae attachment with stuffed stipe.

14. *Oudemansiella* sp. (AGNBP2019-03

Morphological characteristics

Oudemansiella sp. (AGNBP2019-03). Gilled mushroom. *Substrate* dead wood. *Odor* not determined. *Pileus size* 3.4 cm diam. *Pileus surface color* white with yellow ramified setae. *Pileus shape* plane but slightly depressed. *Pileal surface texture* lannate/ waxy but with presence of setae scattered on the surface. *Pileus margin* translucent striate. *Pileal context* white. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe color* white. *Stipe size* 3.7 cm length and 0.6 cm apex diam. *Stipe attachment* central. *Stipe shape* equal. *Stipe surface texture* fibrillose. *Stipe context* white, hollow. *Stipe base* inserted. *Annulus* absent. *Volva* absent.

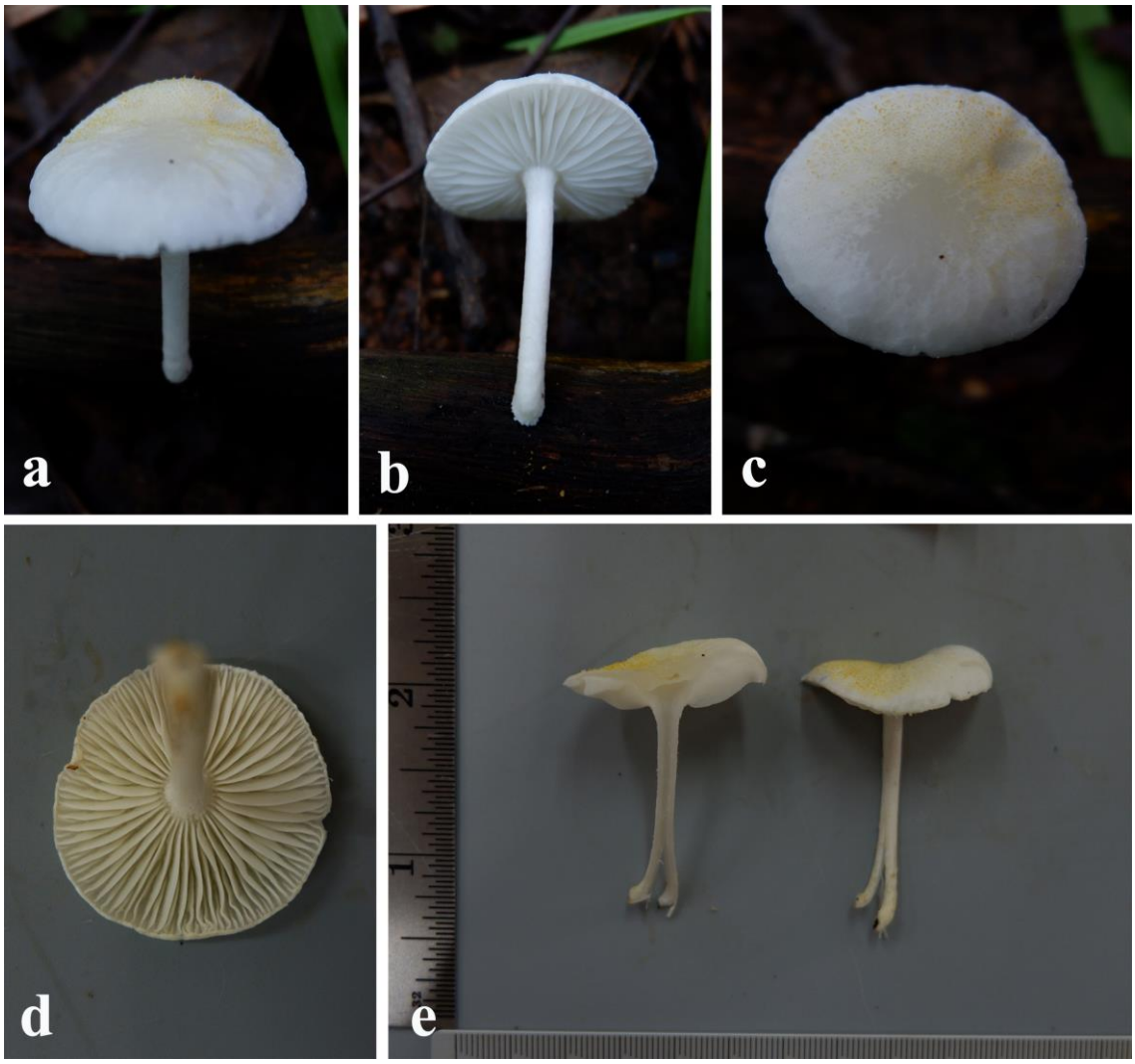

Figure 20. AGNBP2019-03 (*Oudemansiella* sp.) a. basidiome b. Lamellae orientation c. pileus with yellowish ramified setae d. Lamellae orientation e. lamellae attachment to the hollow stipe

15. *Oudemansiella* sp. (AGNBP2019-04)

Morphological characteristics

Oudemansiella sp. (AGNBP2019-04). Gilled mushroom. *Substrate* dead wood. *Odor* not determined. *Pileus size* 6.0 cm diam. *Pileus surface color* yellow-orange. *Pileus shape* plane with slight umbo. *Pileal surface texture* gelatinous, translucent. *Pileus margin* translucent striate. *Pileal context* yellow-orange, gelatinous. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* light yellow-orange. *Lamellulae orientation* regular, no.2. *Stipe color* brown turning darker towards the base. *Stipe size* 2.0 cm length and 0.5 cm apex diam. *Stipe attachment* central. *Stipe shape* slightly tapered. *Stipe surface texture* fibrillose. *Stipe context* brown outward to light yellow towards the center. *Stipe base* inserted. *Annulus* absent. *Volva* absent.

Figure 21. AGNBP2019-04 (*Oudemansiella* sp.) a. basidiome attached to the dead wood b. pileus c. Lamellae orientation d. Lamellae attachment and stipe with narrow canal.

16. *Oudemansiella* sp. (AGNBP2019-05)

Morphological characteristics

Oudemansiella sp. (AGNBP2019-05). Gilled mushroom. *Substrate* dead wood. *Odor* not determined. *Pileus size* 3.0 cm diam. *Pileus surface color* white to light brown towards the center. *Pileus shape* broadly convex. *Pileal surface texture* waxy. *Pileus margin* translucent striate. *Pileal context* white. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe color* light brown to dark brown towards the base. *Stipe size* 2.7 cm length and 0.25 cm apex diam. *Stipe attachment* central. *Stipe shape* equal. *Stipe surface texture* fibrillose. *Stipe context* light brown, hollow. *Stipe base* inserted. *Annulus* absent. *Volva* absent.

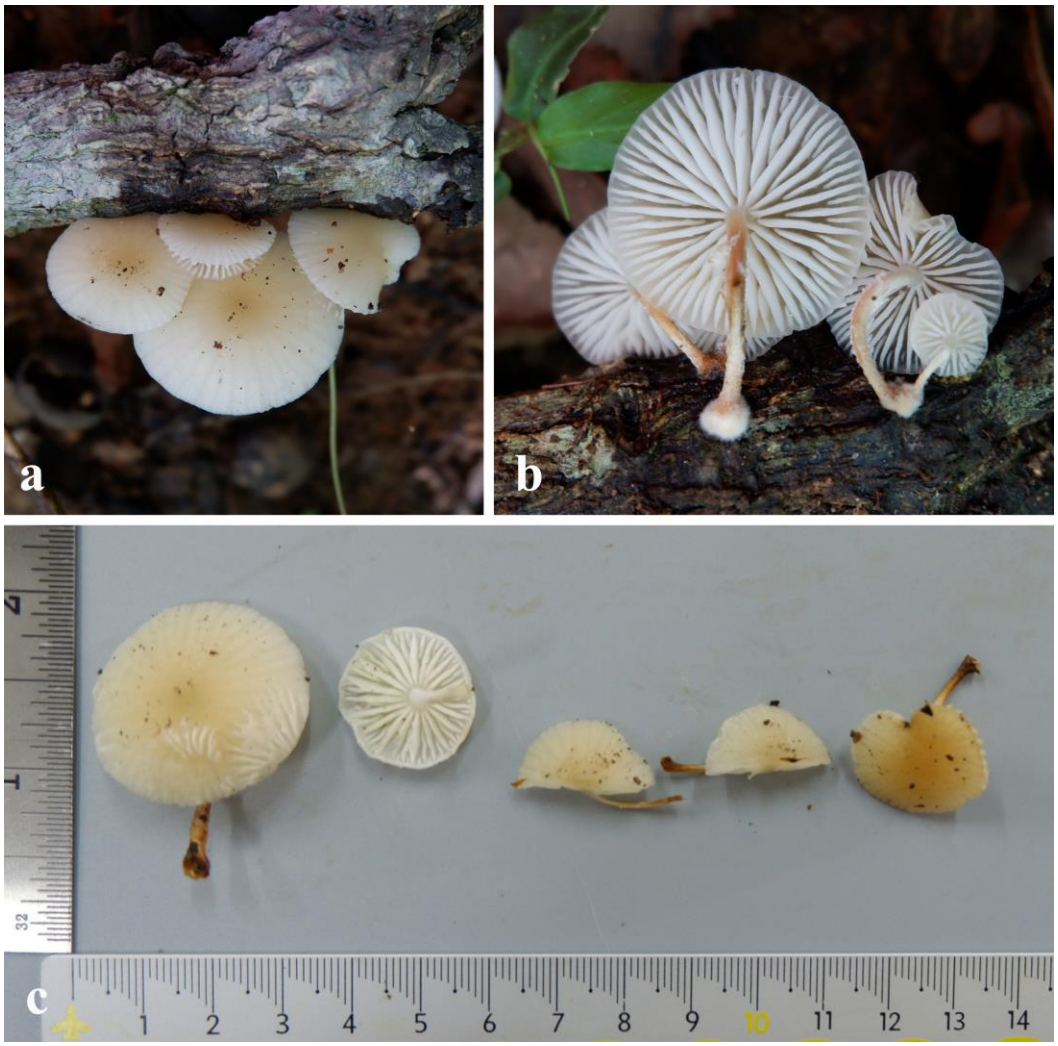

Figure 22. AGNBP2019-05 (*Oudemansiella* sp.) a. Pileus b. Lamellae orientation with stipes attached to the dead wood c. Different orientation of the basidiomes.

17. *Oudemansiella* sp. (AGNBP2019-06)

Morphological characteristics

Oudemansiella sp. (AGNBP2019-06). Gilled mushroom. *Substrate* dead wood. *Odor* not determined. *Pileus size* 2.5-3.0 cm diam. *Pileus surface color* white to light brown towards the center. *Pileus shape* broadly convex, slightly depressed in the center. *Pileal surface texture* waxy. *Pileus margin* translucent striate. *Pileal context* white. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* white. *Lamellulae orientation* regular, no.2. *Stipe color* light brown to dark brown towards the base. *Stipe size* 2.6-2.8 cm length and 0.25 cm apex diam. *Stipe attachment* central. *Stipe shape* equal. *Stipe surface texture* fibrillose. *Stipe context* light brown, hollow. *Stipe base* inserted. *Annulus* absent. *Volva* absent.

Figure 23. AGNBP2019-06 (*Oudemansiella* sp.) a. Pileus b. Lamellae orientation with stipes attached to the dead wood c. Different orientation of the basidiomes.

18. *Oudemansiella* sp. (AGNBP2019-08)

Morphological characteristics

Oudemansiella sp. (AGNBP2019-08). Gilled mushroom. *Substrate* dead wood. *Odor* not determined. *Pileus size* 2.0 cm diam. *Pileus surface color* dark brown from the center to orange-brown towards the margin. *Pileus shape* plane, slightly depressed at the center. *Pileal surface texture* waxy, rubberized. *Pileus margin* translucent striate. *Pileal context* light brown. *Lamellae attachment* adnexed. *Lamellae margin* even. *Lamellae color* light brown. *Lamellulae orientation* regular, no.2. *Stipe color* brown turning darker towards the base. *Stipe size* 1.2 cm length and 0.4 cm apex diam. *Stipe attachment* slightly eccentric. *Stipe shape* equal. *Stipe surface texture* fibrillose. *Stipe context* brown outward to light yellow towards the center, slightly stuffed but with narrow canal. *Stipe base* inserted. *Annulus* absent. *Volva* absent.

Figure 24. AGNBP2019-08 (*Oudemansiella* sp.) a. pileus b. lamellae orientation c. Lamellae attachment and stipe with narrow canal.

B.2 Phylogenetic Analysis

Data I, ITS sequences analysis, a total of one hundred twenty eight sequences consisting of 122 sequences of related species of *Volvariella* from the GenBank, 4 new sequences (PS2018-39, PS2018-42 and PS2018-49 and MFU-M015) of *Volvariella* from Thailand and 2 sequences of outgroup *Pluteus hirtellus and P. umbrosoides*) were included in the analysis (Figure 25).

Data II, a combined nrITS-LSU genes (79 sequences), is composed of 7 new sequences (4 ITS, 3 LSU) and 72 sequences from GenBank (43 ITS, 29 LSU). Totally, there are 59 taxa, and *Pluteus hirtellus and P. umbrosoides* are outgroup.

Both two datasets were aligned using MAFFT version 7.130-win32 (Kahtoh et al., 2002; Kahtoh & Toh, 2008). A Maximum likelihood (ML) analysis was performed in RAxML 7.2.6 (Stamatakis et al. 2008), with GTRGAMMAI as the model of evolution, and branch support was estimated over 1,000 bootstrap partitions (BP) with the rapid bootstrap option.

The results are showed in the Figure 25 and 26. Thai specimens of *Volvariella nivea* (MFU-M015) is clustered with a specimens from China 100%, BS (Figure 25 and 26). Thai specimen of *V. pulla* (PS2018-32) is clustered with Vietnamese specimens 98%, BS in Figure 25, and 100%, BS in Figure 26. Two specimens (PS2018-42 and PS2018-49) are identical in Figure 25 and 26 and this species is related to *V. nivea*, but different in morphology. We are curious that this species should be new species of genus *Volvariella*.

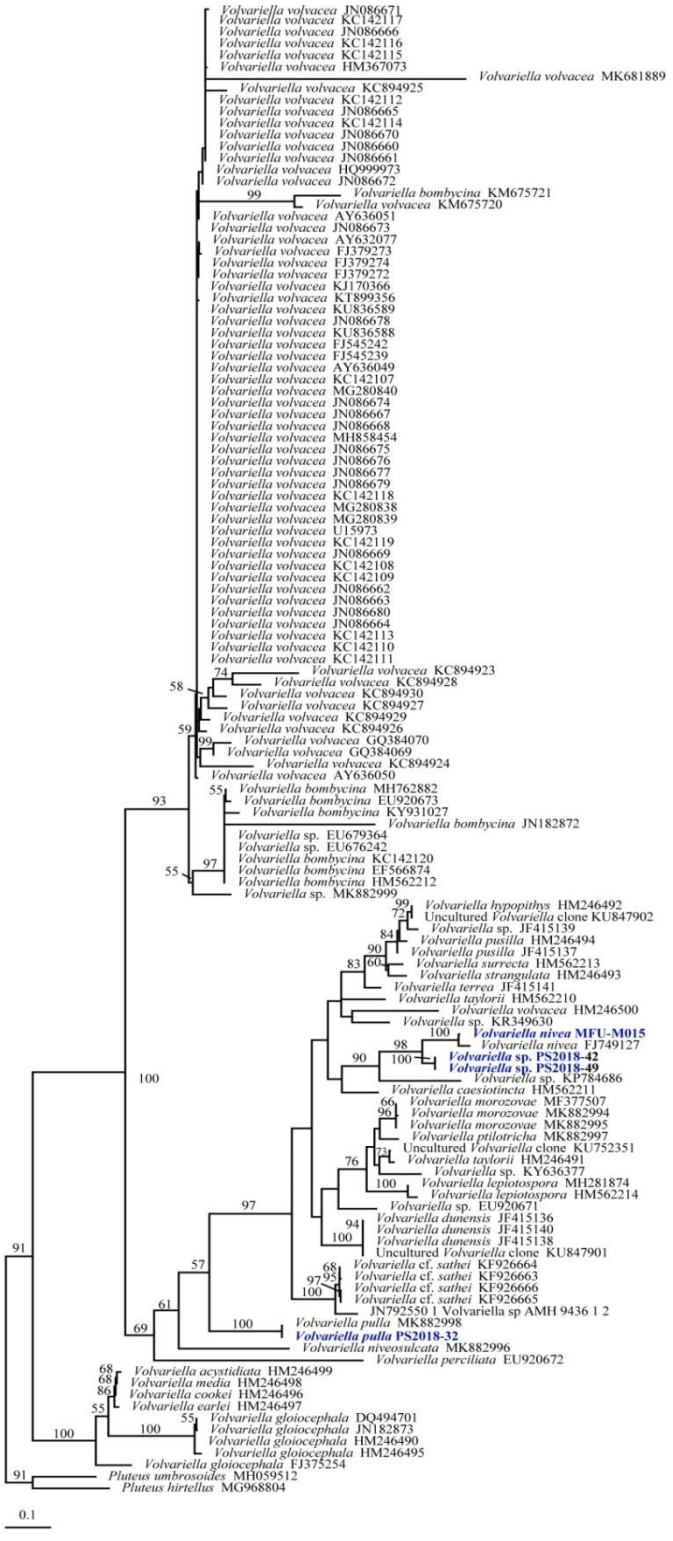

Figure 25. ML of *Volvariella* based on nrITS sequences. GenBank accession numbers are given after species name. Bootstrap values > 50% are indicated above the branch. New Thai sequences

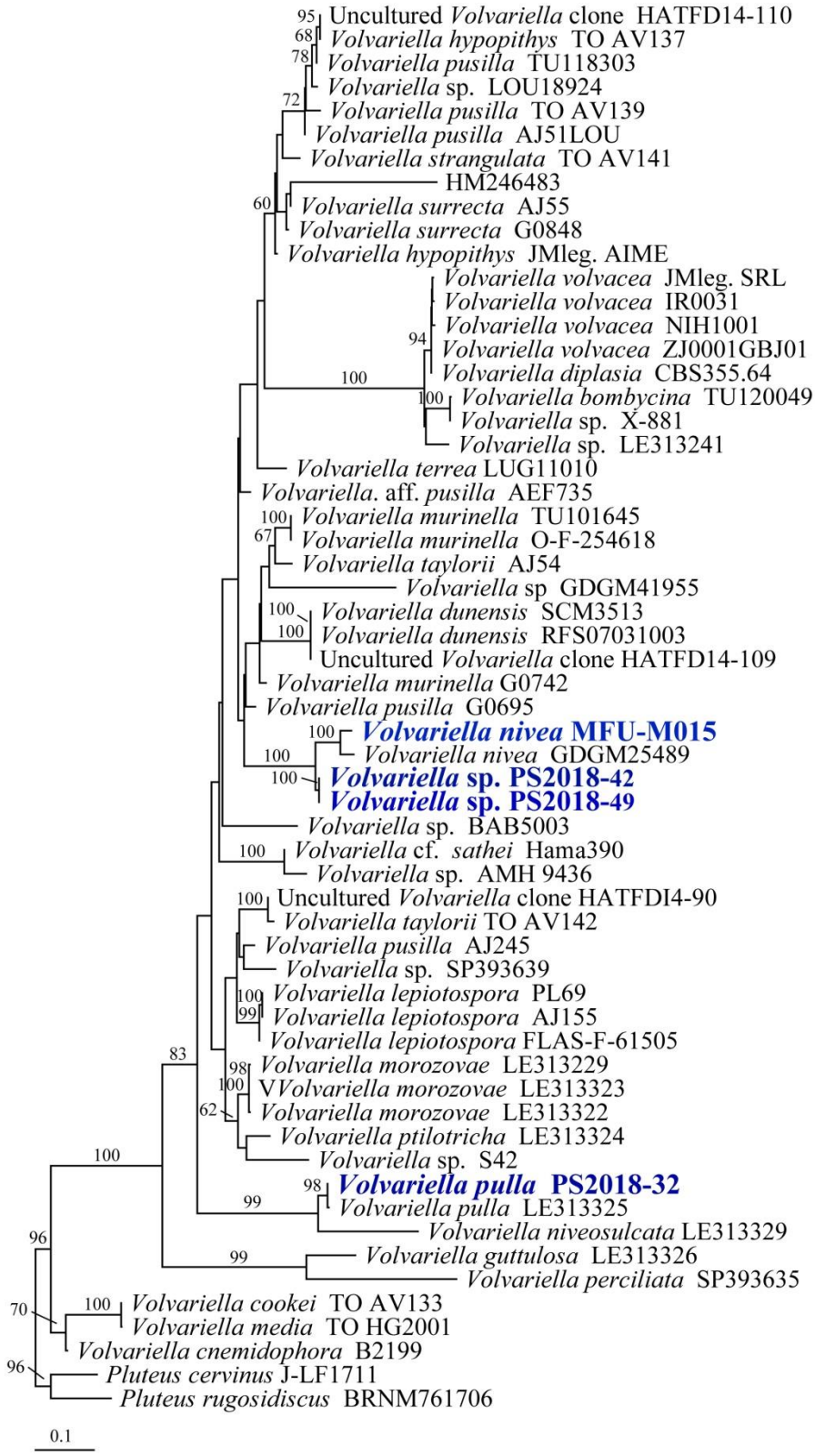

Figure 26. ML of Volvariella based on nrITS-LSU sequences. Herbarium numbers are given after species name. Bootstrap values ≥ 50% are indicated above the branch. New Thai sequences are in blue type. *Pluteus cervinus* (Schaeff.) P. Kumm. and *P. rugosidiscus* Murrill are as outgroup.

C. Growth Optimization

1. Media optimization

1.a. Optimal condition to grow mycelium of *Volvariella* species

A mycelium plug (0.5-mm diameter) of *Volvariella* sp1 was inoculated into media (Potato Dextrose Agar, Corn Meal Agar, Malt Extract Agar and Oat Meal Agar). All cultures were incubated at room temperature for 24 days. *Volvariella* sp1 has the average mycelial growth of 2.8cm on Potato Dextrose Agar, 2.6cm on Corn Meal Agar, 5.3cm on Malt Extract Agar and 3.5cm on Oat Meal Agar. The result showed that among the four media, Malt Extract Agar has the highest yield of mycelial growth of *Volvariella* sp1 and thus will be considered for the later spawn preparation of the said species. *Volvariella volvacea* (Control) has covered the whole plates for all media used after 24 days of incubation at room temperature. However, it has higher mycelial growth density on Malt Extract Agar and Oat Meal Agar. Growth of *Volvariella* sp1 and *Volvariella volvacea* in different media (Figure 27).

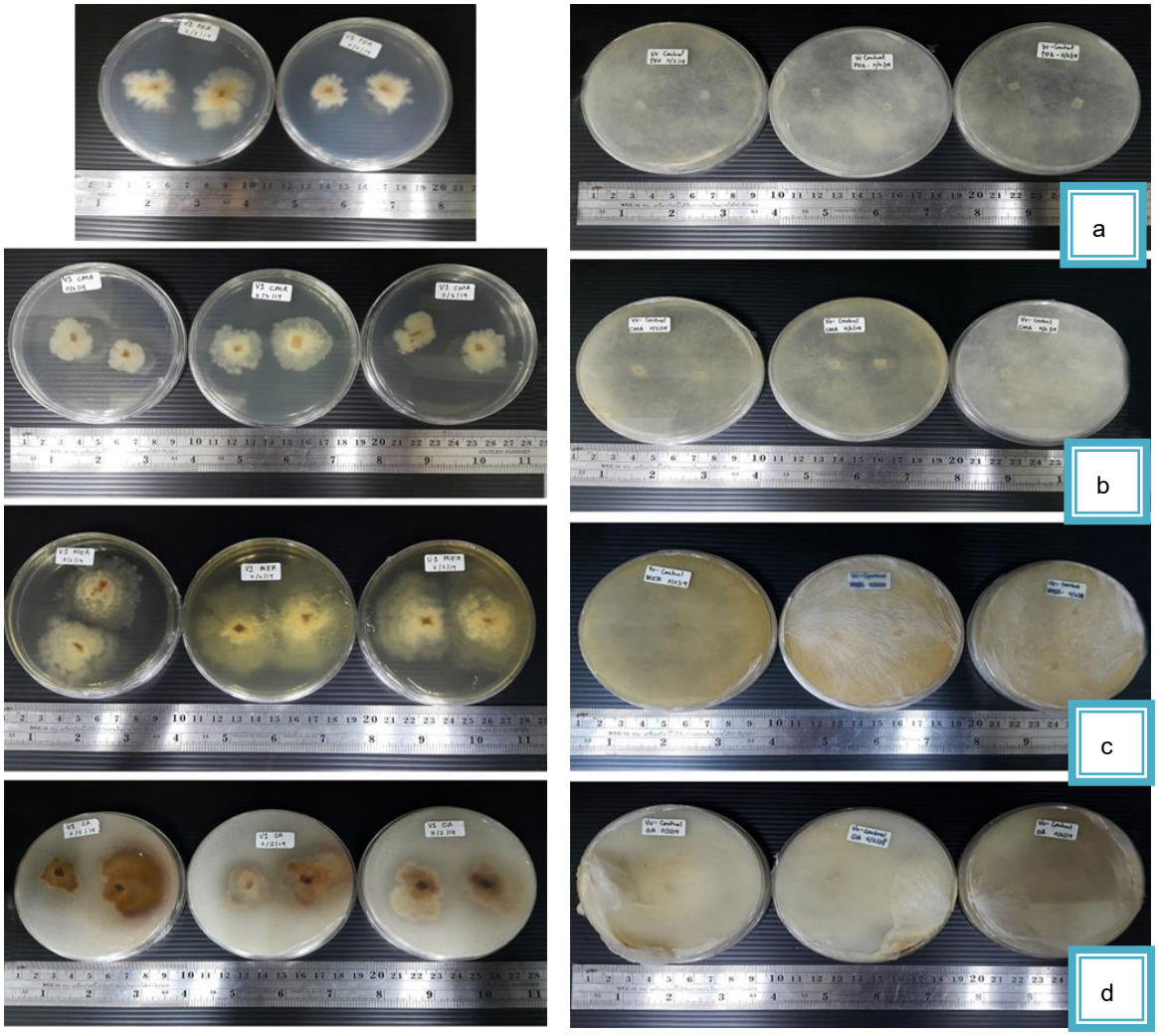

Figure 27. *Volvariella* sp1 (MFU-M015) and *Volvariella volvacea* (Control) growth on different media after 24 days of incubation at room temperature. *Volvariella* strains growth on a) Potato Dextrose Agar b) Corn Meal Agar c) Malt Extract Agar d) Oat Meal Agar.

1.b Optimal condition to grow mycelium of *Xerula* species

Among the 5 different media, Oatmeal Agar has the highest average biomass (0.02398 g), followed by Malt Extract Agar (0.0194 g), Potato Dextrose Agar (0.0081g), Rice Stalk Agar (0.0056) and Corn Meal Agar (0.0034g) after incubated 18 days.

2. Spawn optimization

Four substrates were used for spawn optimization of *Xerula* strain (AGN2019-01) namely sorghum, barley, corn and wheat. Among these substrates sorghum has the highest growth for 8 days (9.8 cm) and fastest mycelial average growth rate (1.23 cm/day), followed by corn (9.08 cm growth; 1.14 cm/day average growth rate), then barley (8.1 cm growth; 1.01 cm/day average growth rate). Wheat substrate has the slowest growth (0.86 cm growth; 0.11 cm/day average growth rate (Fig. 28 and Fig. 29). As for *Volvariella* strain (M015), slow growth has been observed to almost all substrates starting on the 4th day only, however, among them growth in sorghum was the fastest (0.16 cm growth; 0.03 cm/day average growth rate), followed by barley (0.04 cm growth; 0.01cm/day growth rate). No growth on corn and wheat was observed for the *Volvariella* strain (Fig. 30).

Figure 28. *Xerula* sp. growth on different spawn media. a) sorghum b) barley c) corn d) wheat

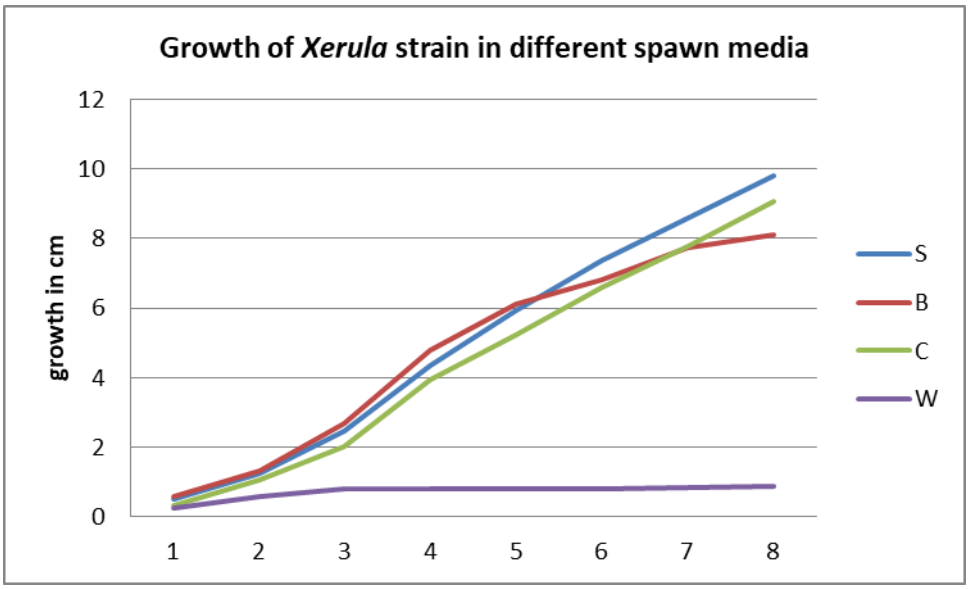

Figure 29. Growth rate of *Xerula* strain (AG2019-01) on different spawn media.

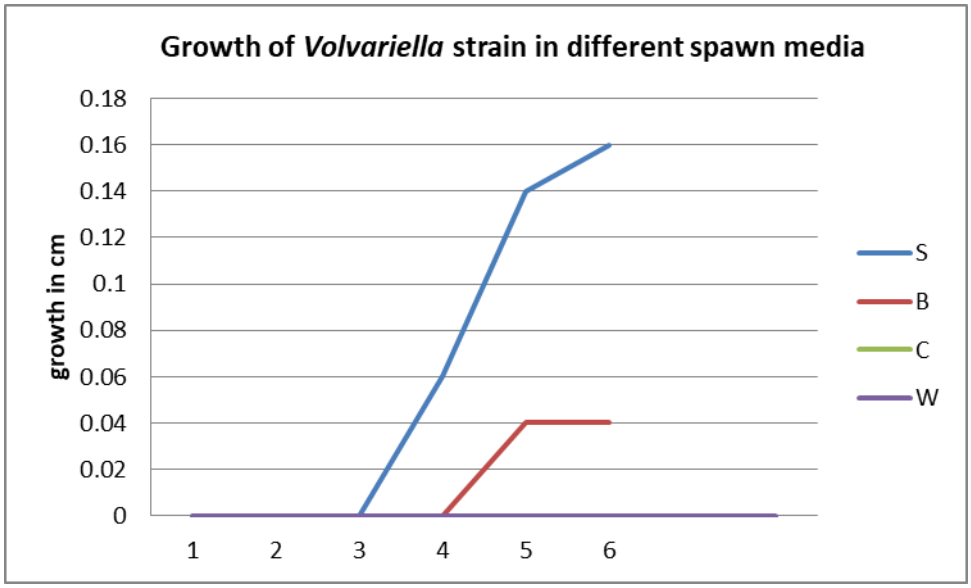

Figure 30. Growth rate of *Volvariella* strain (M015) on different spawn media.

D. Spawning and Cultivation

Sorghum was the ideal substrate in the mycelial growth of *Volvariella* and *Xerula* strains, thus it was used in the preparation of spawns prior to cultivation (Figure 31). However, it was noticed that the growth of *Volvariella* in the bottles with sorghum was slower as compared to the control, thus requiring longer time to prepare the spawn (Figure 31, a and b). The *Xerula* strains have very good growth on sorghum. It only took 3 weeks to prepare the spawn (Figure 31, c).

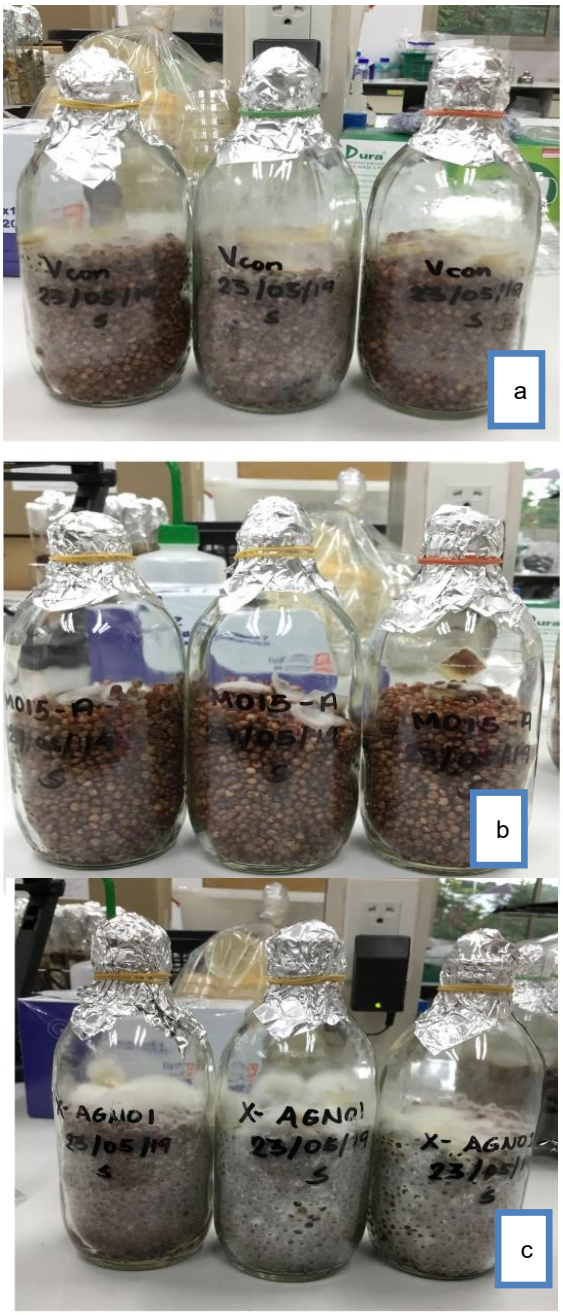

Figure 31. Spawn of *Volvariella* strains in sorghum. a) *Volvariella volvacea* (control) b) *Volvariella* sp. (M015) c) *Xerula* sp. (AGN2019-01)

E. Cultivation

a. Cultivation of *Xerula* strain

Xerula strain was cultivated in 10 bags of rubber sawdusts (Fig 32). Good mycelial growth was observed in each bags. As the primodia appeared the bags were opened to allow growth of the basidiomes. Five out of 10 bags were harvested with fruiting bodies. Fresh and dry weight of each basidiome were noted and samples were processed accordingly. Number of basidiome produced ranged from 1 to 5 fruiting bodies. An average of 46.16g fresh basidiome biomass and 7.46g dry biomass were produced. Fruiting body formation lasted for 4 weeks.

Figure 32. a-b, Basidiomes of *Xerula* sp. grown on saw dust bags

b. Cultivation of *Volvariella* strain

Cultivation of *Volvariella* sp.(M015) was done using rice straws on trays (Fig. 33). However, no growth was observed on trays since the set-up was contaminated by other hypomycetes. In the control, only two fruiting bodies were harvested in the duration of 2 weeks (Fig. 34). Slow growth of mycelia was also observed on trays.

Figure 33. Cultivation of 4 *Volvariella* strains using rice straws (with and without fertilizer

Figure 34. Basidiomes of *Volvariella* harvested from the set-up.

F. Extraction of bioactive compounds

Strains of *Volvariella* and *Xerula* were grown in the liquid medium (Malt Extract Broth) for 30 days. Extraction was carried out using established protocols (Thongbai et al. 2013). Mycelia grown on solid medium were also sent to laboratory for analysis of bioactivities.

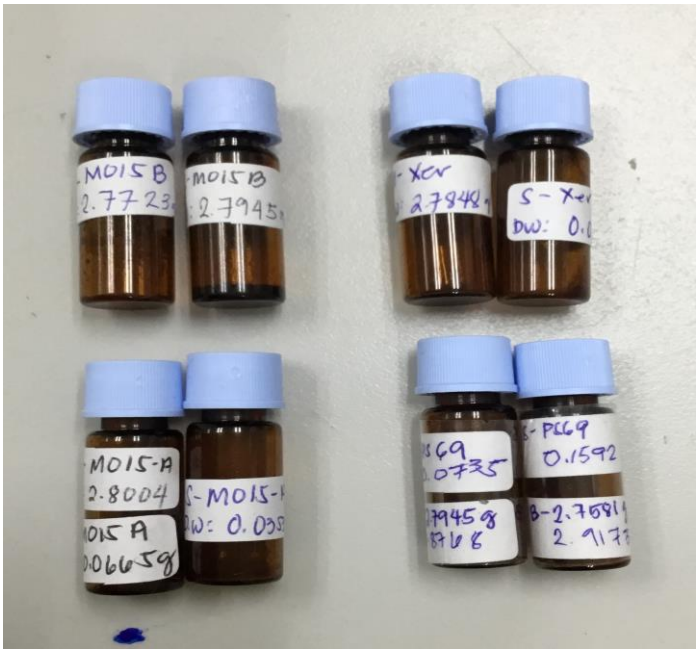

Figure 35. Crude extracts of *Volvariella* and *Xerula* strains.

Output

1) Publications

A: Hyde, K.D et al. (2018).Thailand's amazing diversity: up to 96% of fungi in northern Thailand may be novel. Fungal Diversity 93: 215. https://doi.org/10.1007/s13225-018-0415-7

Fungal Diversity (2018) 93:215–239
https://doi.org/10.1007/s13225-018-0415-7

Thailand's amazing diversity: up to 96% of fungi in northern Thailand may be novel

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Abstract
Fungi have been often neglected, despite the fact that they provided penicillin, lovastatin and many other important medicines. They are an understudied, but essential, fascinating and biotechnologically useful group of organisms. The study of fungi in northern Thailand has been carried out by us since 2005. These studies have been diverse, ranging from ecological aspects, phylogenetics with the incorportation of molecular dating, taxonomy (including morphology and chemotaxonomy) among a myriad of microfungi, to growing novel mushrooms, and DNA-based identification of plant pathogens. In this paper, advances in understanding the biodiversity of fungi in the region are discussed and compared with those further afield. Many new species have been inventoried for the region, but many unknown species remain to be described and/or catalogued. For example, in the edible genus *Agaricus*, over 35 new species have been introduced from northern Thailand, and numerous other taxa await description. In this relatively well known genus, 93% of species novelty is apparent. In the microfungi, which are relatively poorly studied, the percentage of novel species is, surprisingly, generally not as high (55–96%). As well as Thai fungi, fungi on several hosts from Europe have been also investigated. Even with the well studied European microfungi an astounding percentage of new taxa (32–76%) have been discovered. The work is just a beginning and it will be a daunting task to document this astonishingly high apparent novelty among fungi.

Keywords *Agaricus* · *Amanita* · *Colletotrichum* · *Cornus* · Fungal diversity · Pandanaceae · Rosaceae · *Rosa* · Teak fungi

Introduction

Fungi are an incredibly understudied, but an essential, fascinating and biotechnologically useful group of organisms. The fungi of northern Thailand have been studied by Hyde and coworkers since 2005. The studies have been diverse, ranging across ecology, traditional taxonomy, phylogenetics, evolution, microbial community and chemotaxonomy (Thongkantha et al. 2008; Pinnoi et al. 2010; Phookamsak et al. 2015; Wurzbacher et al. 2017; Norphanphoun et al. 2018; Tedersoo et al. 2018), to growing novel mushrooms (Thongklang et al. 2014), molecular identification of endophytes and plant pathogens (Jayawardena et al. 2016b; Doilom et al. 2017b), and identification of entomophagous fungi (Xiao et al. 2017, 2018).

Although there are many negative facets to fungi (see Hyde et al. 2018), they are an essential component of most ecosystems and without them there would be ecological imbalance, and possibly mankind would not survive on earth (Watkinson et al. 2015). They are major contributors to nutrient cycling, and the main organisms which can degrade lignocellulose in wood and leaves (Pointing et al. 2005; Bucher et al. 2004; Tang et al. 2005); without them we would live amongst mountains of dead trees (Gadd et al. 2007). Many species exist as symbionts with plants

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are finding that more than 93% of species collected are new to science. In the microfungi which appear to be relatively poorly studied, the percentage does not appear to be as high. The studied regions mainly includes three provinces in northern Thailand. The southern, eastern and central provinces of Thailand and surrounding countries of Cambodia, Myanmar, Laos and Vietnam have barely been studied for fungi and thus we predict that there are huge numbers of new species waiting to discovered in this region. At the same time, we have been finding ways to exploit these fungi. Our work has resulted in the discovery of at least ten new species which are being developed as novel industrial mushrooms. We have also isolated at least ten novel medicinal compounds from Thai fungi and are also looking at ways to exploit them in biocontrol. All of the fungi mentioned above are known to produce various therapeutic metabolites with high biological activities. It is therefore very important to properly characterize not only these compounds, but to carefully resolve the species names, so that researchers can better identify and screen potential taxa for future biotechnological applications. Fungi have been poorly exploited and yet have a huge potential in biocontrol, bioremediation, novel compound discovery as well as basic industrial organisms as edible mushrooms, fertilizers and cosmetics. With such high novelty, there is a need for extensive research to exploit the biotechnological potential of these fungi.

Acknowledgements K.D. Hyde would like to thank the Thailand Research Fund for the grant ''Domestication and bioactive evaluation of Thai *Hymenopellis*, *Oudemansiella*, *Xerula* and *Volvariella* species (basidiomycetes)" Grant No. as : DBG6180033 for funding this work. Mae Fah Luang University for the grant ''Taxonomy diversity, phylogeny and evolution of fungi in Capnodiales" (Grant No: 666713), for supporting this study. The authors extend their appreciation to the German Academic Exchange Service (DAAD) for a joint TRF-DAAD [PPP 2017–2018] academic exchange grant to K.D. Hyde and M. Stadler. Mingkwan Doilom thanks grant for postdoctoral researchers funded by Yunnan Human Resources and Social Security Department, and The 64th Grant from China Postdoctoral Science Foundation.

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B: Hyde KD et al. (2019) The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Diversity 97: 1–136. https://doi.org/10.1007/s13225-019-00430-9

Fungal Diversity
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REVIEW

The amazing potential of fungi: 50 ways we can exploit fungi industrially

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Abstract
Fungi are an understudied, biotechnologically valuable group of organisms. Due to the immense range of habitats that fungi inhabit, and the consequent need to compete against a diverse array of other fungi, bacteria, and animals, fungi have developed numerous survival mechanisms. The unique attributes of fungi thus herald great promise for their application in biotechnology and industry. Moreover, fungi can be grown with relative ease, making production at scale viable. The search for fungal biodiversity, and the construction of a living fungi collection, both have incredible economic potential in locating organisms with novel industrial uses that will lead to novel products. This manuscript reviews fifty ways in which fungi can potentially be utilized as biotechnology. We provide notes and examples for each potential exploitation and give examples from our own work and the work of other notable researchers. We also provide a flow chart that can be used to convince funding bodies of the importance of fungi for biotechnological research and as potential products. Fungi have provided the world with penicillin, lovastatin, and other globally significant medicines, and they remain an untapped resource with enormous industrial potential.

Keywords Biocontrol · Biodiversity · Biotechnology · Food · Fungi · Mushrooms

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basidimycete culture, which was obtained in very high yields in a relatively short time, owing to the fact that modern bioprocess technology and methods of systems biology were employed.

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C: Paper Review (revision on going)

Important species of *Oudemansiella*, *Xerula, Mucidula* and *Hymenopellis* (Agaricales: Physalacriaceae) as sources of bioactive compounds: A review

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Abstract

The genera *Oudemansiella, Xerula, Mucidula* and *Hymenopellis* are groups of promising mushrooms that can be great sources of bioactive compounds. Numerous studies showed many bioactivities of their extracts such as anti-oxidative, anti-cancer, antimicrobial and anti-inflammatory. Novel bioactive compounds isolated were dominated by oudemansins and strobilurins and their biological activities were recognized. To determine the sustainability of these mushrooms as stable source, their taxonomy, distribution and cultivation were also discussed. As confirmed in the different studies, these groups of Agaricales mushrooms are quite important source of bioactive compounds with bright future in the market in the nutraceutical, pharmaceutical and medicinal arena. Moreover, the active compound, strobilurin, has already made its name in the market as potent fungicides. There are also challenges that need to be addressed in order to fully exploit the potential of these genera. Thus, further studies are recommended to isolate high quality bioactive compounds and fully understand their mode of actions.

Keywords: anti-oxidative, anti-cancer, antimicrobial, anti-inflammatory, bioactive compounds

Utilization from the research project

-

2) Book and patent (if any)

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Appendix