MYCOBIOLOGY
2024, VOL. 52, NO. 1, 1–12
https://doi.org/10.1080/12298093.2023.2295633

RESEARCH ARTICLE

OPEN ACCESS

A New Edible Wild Mushroom Species, *Panus sribuabanensis* (Panaceae, Polyporales) from Northern Thailand and Its Nutritional Composition, Total Phenolic Content, and Antioxidant Activity

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ABSTRACT

A new edible wild mushroom species, described herein as *Panus sribuabanensis*, was collected from local markets and natural forests located in northern Thailand. This species is characterized by its medium to large-sized basidiomata, broadly ellipsoid to ellipsoid-shaped basidiospores, dimitic hyphal system, and the absence of hyphal pegs. A molecular phylogenetic analysis of combined the internal transcribed spacer (ITS) and large subunit (nrLSU) of nuclear ribosomal DNA sequences supported the monophyly of *P. sribuabanensis* as a distinct lineage within the genus *Panus*. Full description, illustrations, color photographs, and a phylogenetic tree to show the placement of *P. sribuabanensis* are provided. The dried mushroom showed a nutritional composition within the range of 2.58%–2.67% for fat content, 27.10%–27.98% for protein, and 43.97%–44.10% for carbohydrates. The ethanolic extracts from this mushroom exhibited a total phenolic content ranging from 0.66 to 0.74 mg GAE/g dry weight (dw). Moreover, the antioxidant activities of ethanolic extracts evaluated by the 2,2-diphenyl-1-picrylhydrazyl (0.90–1.08 mg TE/g dw) and ferric reducing antioxidant power (0.93–1.08 mg TE/g dw) assays demonstrate higher activity compared to the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay (0.44–0.51 mg TE/g dw). The outcomes of this study provide significant information on the nutritional value, phenolic content, and antioxidant activity potential of this new mushroom species discovered in northern Thailand.

ARTICLE HISTORY
Received 18 September 2023
Revised 29 November 2023
Accepted 12 December 2023

KEYWORDS
Edible mushroom; nutritional value; phylogeny; saprotrophic mushroom; taxonomy

1. Introduction

As edible wild mushrooms contain essential minerals, nutrients, and vitamins, they are well-known to be excellent sources for humans [1–4]. These mushrooms are thought to be sources of numerous bioactive substances with a variety of beneficial biological effects, including antidiabetic, anti-inflammatory, antimicrobial, anticancer, antioxidant, and immunomodulatory properties [1,3,5–7]. Additionally, edible wild mushrooms have been recognized for their important role in the food security and medicinal use of ethnic groups and tribes all over the world [8,9]. Every year, numerous species of edible wild mushrooms are collected in northern Thailand, which are abundant during the rainy period, especially from mid-May through October. Local farmers have collected them in forests for general consumption and sell them at localized, roadside, and urban markets [10–12]. According to preliminary investigations conducted in northern Thailand, numerous genera of edible wild mushrooms have been reported, including *Agaricus*, *Amanita*, *Astraeus*, *Boletus*, *Cantharellus*, *Lactarius*, *Lentinus*, *Panus*, *Pleurotus*, *Russula*, and *Termitomyces* [11–13]. However, Thai edible wild mushrooms have not yet been well studied and documented. Some species have only been described based on morphological characteristics that presumably correspond to previously known species in America and Europe [14,15]. As a result, several edible wild mushrooms in northern Thailand have been misidentified due to insufficient information and an absence of molecular data. Therefore, the correct identification of these edible mushroom species currently requires the combination of morphological and genetic data.

Fries [16] first described the genus *Panus* in 1838, and *P. conchatus* is considered the type species. This

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genus is well-known for growing on dead or decaying wood and is found throughout the world in subtropical and tropical regions [17–20]. The main characteristics of *Panus* species are free gills, highly radiating hymenophoral trama, frequently dimitic hyphae, and lack of hyphal pegs [16,20,21]. In 1981, Corner separated the genera *Lentinus* and *Panus* based on their different hyphal systems [21]. *Lentinus* distinguished from *Panus* by its radiating, descending, or intermediate hymenophoral trama, trimitic hyphae, and hyphal pegs. However, Pegler [22] recognized *Panus* as a subgenus of *Lentinus*. Later, several studies confirmed that *Lentinus* and *Panus* are separate genera [19,23]. Additionally, recent phylogenetic studies of the order Polyporales confirmed that *Panus* and *Lentinus* are separate genera. *Lentinus* belongs to the family Polyporaceae, while *Panus* has been placed in the family Panaceae [24–26]. Currently, the family Panaceae contains two genera, namely *Cymatoderma* and *Panus* [26,27]. According to Wijayawardene et al. [27], there are 20 species of *Panus* worldwide, although there are about 99 recorded entries in the Index Fungorum (http://www.indexfungorum.org, accessed on July 10, 2023). Although, these records can include many misidentifications and some species have not yet been well-documented. Moreover, there can also be a lack of comprehensive molecular data. Furthermore, some *Panus* species, namely *P. conchatus*, *P. lecomtei* (syn. *P. neostrigosus* and *L. strigosus*), *P. rudis*, and *P. strigellus*, are typically considered as edible species [28–30].

Prior to this present study, six *Panus* species, including *P. ciliatus*, *P. conchatus*, *P. luteolus*, *P. roseus*, *P. similis*, and *P. tephroleucus*, have been discovered in Thailand [31,32]. According to an investigation of the diversity of mushrooms in forests and local markets in northern Thailand in 2020, we discovered an interesting edible species of *Panus* that had not previously been described. Therefore, the aim of this study was to identify the collected *Panus* specimens using morphological data and phylogenic analyses of the combined ITS and nrLSU sequences data. The collected specimens were thoroughly documented, including detailed descriptions, color photographs, line-drawing illustrations, and the phylogenetic tree. Herein, the nutrient composition, total phenolic content, and antioxidant activity of this mushroom were examined.

2. Materials and methods

2.1. Sample collection

During the rainy season of 2020, basidiomata were collected from local marketplaces and natural forests located in Lamphun Province, northern Thailand and stored in a plastic box for transport to the laboratory. After that, specimens were dried completely in a hot air oven at 45 °C. Each dried specimen was deposited at the Herbarium of Sustainable Development of Biological Resources within the Faculty of Science at Chiang Mai University (SDBR-CMU) in Thailand. Additionally, MycoBank number was provided.

2.2. Morphological observation

Macromorphological data were described based on fresh specimens within 24 h. The color name and code were followed according to Korner and Wansher [33]. Sections of the dried specimens were mounted using a 5% aqueous solution of potassium hydroxide (KOH). The 1% aqueous Congo red solution and Melzer's reagent were used to increase the contrast of structures and check the amyloid reaction of basidiospores. The microscopic features (basidia, basidiospores, cystidia, and hyphae) were carried out using a light Eclipse Ni U microscope (Nikon, Tokyo, Japan). The Tarosoft (R) Imaging Frame Work program was employed to calculate the size of each microscopic feature based on at least 50 measurements. The notation [n/m/p] represents the number of basidiospores 'n' measured from 'm' basidiomata of 'p' collections. Basidiospore statistics are expressed as (a–)b–c(–d), where 'a' and 'd' are the extreme values, and 'b–c' is the range comprising 95% of all values. Additionally, the average basidiospore length (L') and width (W') were reported. The Quotient (Q) for basidiospores was calculated by dividing the length by the width of each individual basidiospore, and Q_m was obtained by taking the average of these Q values from all the measured basidiospores ± standard deviation. The terminology of Largent et al. [34] was used to describe microscopic features.

2.3. DNA extraction, PCR amplification, and sequencing

The FAVORGEN DNA Extraction Mini-Kit (Ping-Tung, Taiwan) was being used to extract genomic DNA from the tissue inside of fresh specimens. Polymerase chain reactions (PCR) were performed to amplify the internal transcribed spacer (ITS) and large subunit (nrLSU) genes of the nuclear ribosomal DNA using ITS5/ITS4 [35] and LR0R/LR5 [36] primers, respectively. Two separate PCR reactions were conducted to amplify these two domains, each commencing with an initial denaturation step for 5 min at 95 °C. Following this, 35

cycles were performed using a peqSTAR thermal cycler (PEQLAB Ltd., UK), with each cycle consisting of denaturation for 30 s at 95 °C, annealing for 45 s at 52 °C, and an extension for 1 min at 72 °C, and a final extension for 10 min at 72 °C. The PCR products were examined through 1% agarose gel electrophoresis and subsequently purified using the NucleoSpin Gel and PCR Clean-up Mini kit (Macherey-Nagel, Düren, Germany). The PCR products were then sequenced through the Sanger sequencing approach at 1st Base Company in Malaysia.

2.4. Sequence alignment and phylogenetic analyses

A BLASTn search was performed on the sequences by uploading them to GenBank (http://blast.ncbi.nlm.nih.gov, accessed on June 25, 2023). Sequences from previous studies, GenBank database (with ≥ 85–100% sequence similarity and ≥ 60% query coverage), and this study were selected and are shown in Table 1. MUSCLE [37] was used for multiple sequence alignment, and alignments were checked and edited manually. The finalized alignment of the concatenated ITS and nrLSU sequences was provided to TreeBASE (https://www.treebase.org/) with a submission ID of 30689.

The combined dataset of ITS and nrLSU was used for phylogenetic analysis. A phylogenetic tree was reconstructed using both maximum likelihood (ML) and Bayesian inference (BI) approaches. *Lentinus polychrous* and *Polyporus thailandensis* were selected as the outgroup. The GTRCAT model, which consists of 25 categories and 1000 bootstrap (BS) replications, was used in the ML analysis, which was carried out on the CIPRES online platform using RAxML-HPC2 version 8.2.10 [38]. MrBayes version 3.2.6 was carried out the BI analysis [39,40]. For the BI analysis, the evolutionary model of nucleotide substitution was individually selected for each gene region based on the Akaike Information Criterion (AIC) using jModeltest 2.1.10 [41]. GTR + I + G was the best-fit model for ITS and nrLSU. The posterior probabilities (PPs) for the BI analysis were estimated using Markov chain Monte

Table 1. Details of sequences used in molecular phylogenetic analyses in this study.

Fungal taxa	Isolate/voucher	Origin	GenBank accession number	
			ITS	nrLSU
Cerrena aurantiopora	NIBR FG0000102423[T]	South Korea	NR158290	NG060384
C. unicolor	FD-299	USA	KP135304	KP135209
C. zonata	Cui 18502	China	ON417154	ON417204
C. zonata	CLZhao 7076	China	OM955814	–
Cymatoderma caperatum	MES-3721	USA	ON383384	–
Cy. elegans	Dai 17511	China	ON417155	ON417205
Cy. elegans	CBS 491.76	Japan	JN649340	JN649340
Lentinus polychrous	MFLU22 0030	Thailand	OM780266	OM802487
Panus bambusinus	AK61b[T]	India	MW453097	–
P. ciliatus	SP446150	Brazil	MT669118	MT669140
P. ciliatus	FB11755	USA	–	AY616008
P. conchatus	CBS 267.58	Germany	MH869312	MH857778
P. conchatus	X1234	Finland	JN710579	JN710579
P. conchatus	KUMCC18-0047	China	MK192053	MK333258
P. conchatus	LE265028	Russia	KM411463	KM434323
P. neostrigosus	LSPQ-NSM-106	Canada	KU761234	KU761114
P. neostrigosus	LSPQ-NSM-107	Canada	KU761235	KU761115
P. neostrigosus	LSPQ-NSM-108	Canada	KU761236	KU761116
P. parvus	URM80840	Brazil	MT669125	MT669145
P. purpuratus	MK404671	New Zealand	MK404671	–
P. roseus	HKAS 94714	China	KY490136	–
P. rudis	ZJ1005DKJ02	China	KU863049	AF287878
P. rudis	ZJ1005DKJ03	China	KU863050	–
P. rudis	ZJ1005DKJ04	China	KU863051	–
P. similis	UOC SIGWI S38	Sri Lanka	KR818820	–
P. similis	KWGM 39	India	KY630517	–
P. similis	LE287548	Vietnam	KM411466	KM411482
P. strigellus	B6	Paraguay	MW407012	–
P. strigellus	INPA239979	Brazil	JQ955724	JQ955731
P. strigellus	INPA243940	Brazil	JQ955725	–
P. tephroleucus	CMINPA 1860	Brazil	MN602052	–
P. sribuabanensis	**SDBR-CMUNK0924**	**Thailand**	**OR447474**	**OR447383**
P. sribuabanensis	**SDBR-CMUNK0930**	**Thailand**	**OR447475**	**OR447384**
P. sribuabanensis	**SDBR-CMUNK0931[T]**	**Thailand**	**OR447476**	**OR447385**
P. sribuabanensis	**SDBR-CMUNK0940**	**Thailand**	**OR447477**	**OR447386**
P. sribuabanensis	**SDBR-CMUNK1100**	**Thailand**	**OR447478**	**OR447387**
Polyporus thailandensis	MSUT 6734[T]	Thailand	NR155033	LC052219
Radulodon casearius	Cui 17979	China	ON417185	ON417236
R. casearius	HHB9567	USA	KY948752	KY948871
R. yunnanensis	BJFC 010487[T]	China	NR182985	–

Superscript "T" represents type specimen. "–" represents the absence of sequence data in GenBank. The sequences obtained in this study are bold.

Carlo sampling (MCMC). Tree samples were taken every 1000th generation during the one million generation run of six simultaneous Markov chains. At the end of the run, an average standard deviation of split frequencies equaled 0.00864. The remaining trees were utilized to calculate PPs in the majority-rule consensus tree after the first 25% of produced trees representing the burn-in phase of the analysis were discarded. Significant support was given to branches with bootstrap support (BS) and PP values greater than or equivalent to 70% and 0.95, respectively [42, 43]. For visualization of tree topologies, FigTree version 1.4.0. was used. Moreover, pairwise genetic distances between closely related species were calculated using MEGA version 6 [44].

2.5. Nutritional analysis

A total of three specimens SDBR-CMUNK0924, SDBR-CMUNK0930, and SDBR-CMUNK1100 were used because their dry weights were sufficient for the test. Each dried specimen was grinded through the Waring blender (New Hartford, CT, USA). The determination of nutritional content (including ash, carbohydrate, fat, fiber, and protein) following to the official procedure established by the Association of Official Analytical Chemists (AOAC) [45] at the Central Laboratory, Company Limited, Chiang Mai, Thailand.

2.6. Preparation of mushroom extracts

The preparation of mushroom extract followed the processes mentioned by Kaewnarin et al. [46]. Each dried specimen (10 g powder) was extracted using 100 mL of absolute ethanol at 25 °C with shaking (150 rpm) on a reciprocal shaker. Following a 24-hour period, each extract was subjected to an ultrasonic bath (Elma Transsonic Digital, Singen, Germany) for 3 h at 60 °C and subsequently filtered using Whatman's No. 1 filter paper. After that, the residue was extracted twice with absolute ethanol as previously described. The ethanol-based extract was dehydrated using a rotary evaporator at a temperature of 40 °C. The crude extract was dissolved in 100 mL of absolute ethanol and stored at −20 °C until further studies on a total phenolic content and antioxidant property.

2.7. Determination of total phenolic content

The Folin-Ciocalteu assay was used for measuring the total phenolic content [47]. A volume of 0.25 mL of mushroom extract was combined with 2.5 mL of deionized water and 0.5 mL of the Folin-Ciocalteu reagent. After being incubated for 5 min, 0.5 mL of 20% (*w*/*v*) Na_2CO_3 was added into the mixture, and the solution was then placed in a dark for 1 h at 25 °C. The absorbance at 760 nm was measured. The total phenolic content in the samples was determined by employing a gallic acid standard curve for calculation. The results were reported as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g dw). Five replicates of each sample extract were performed.

2.8. Antioxidant assay

In this study, the antioxidant activity of the mushroom extracts was assessed using three distinct methods, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays. The DPPH, ABTS, and FRAP assays were carried out in accordance with the procedures described in the previous studies by Kaewnarin et al. [46], Re et al. [48], and Li et al. [49], respectively. Trolox was employed as a reference compound. The trolox equivalent antioxidant capacity per gram of dry weight (TE/g dw) was used to represent the DPPH, ABTS, and FRAP activities. Five replicates of each sample extract were performed.

2.9. Statistical analysis

The Tukey's test was used to determine significant differences at the $p < 0.05$ level following one-way analysis of variance (ANOVA) of the data with the SPSS 16.0 software. Additionally, the SPSS program was used to calculate the Pearson correlation coefficients (r) of the total phenolic content and the antioxidant activity of the extract at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Phylogenetic analyses

In this study, five *Panus* specimens were collected from Lamphun Province, Thailand. Three specimens (SDBR-CMUNK0931, SDBR-CMUNK0940, and SDBR-CMUNK1100) were collected from natural forests, while two specimens (SDBR-CMUNK0924 and SDBR-CMUNK0930) were from a local market. The aligned matrix of the combined gene dataset consisted of 1605 characters including gaps (1–722 bp for ITS and 723–1605 bp for nrLSU). Sequence alignment has 575 distinct alignment patterns with 35.19% of undetermined characters or gaps. The

final ML optimization likelihood value obtained from the RAxML analysis was −6746.850673. The topologies of the phylogenetic trees were similar according to ML and BI analyses. As a result, a phylogenetic tree generated from the ML analysis is shown in Figure 1. The phylogenetic tree was consistent with the results of previous phylogenetic studies [24,25]. Phylogenetic analyses assigned the genera *Cymatoderma* and *Panus* in the family Panaceae. It was found that five specimens obtained in this study formed a monophyletic clade within the genus *Panus* (BS = 100% and PP = 1.0) and clearly separated them from the previously known species of *Panus*. These five specimens, introduced as *P. sribuabanensis*, formed a sister taxon to *P. bambusinus* and *P. purpuratus* with 75% BS and 0.98 PP support values.

3.2. Taxonomic description

Panus sribuabanensis J. Kumla, N. Suwannarach & S., Lumyong sp. nov. Figure 2.

MycoBank number: MB849810

Diagnosis: Distinguishable from *P. bambusinus* by its narrower basidiospores and wider cells of cheilocystidia.

Etymology: *'sribuabanensis'* is named of Sri Bua Ban Subdistrict, Lamphun Province where type species was collected.

Holotype: THAILAND, Lamphun Province, Mueang District, Sri Bua Ban Subdistrict, Chiang Mai University Haripunchai Campus, (18°30'18"N 99°8'24"E, elevation 400 m), on decaying wood in a tropical deciduous forest, 16 August 2020, J. Kumla and N. Suwannarach, SDBR-CMUNK0931, gene sequences OR447476 (ITS) and OR447385 (nrLSU).

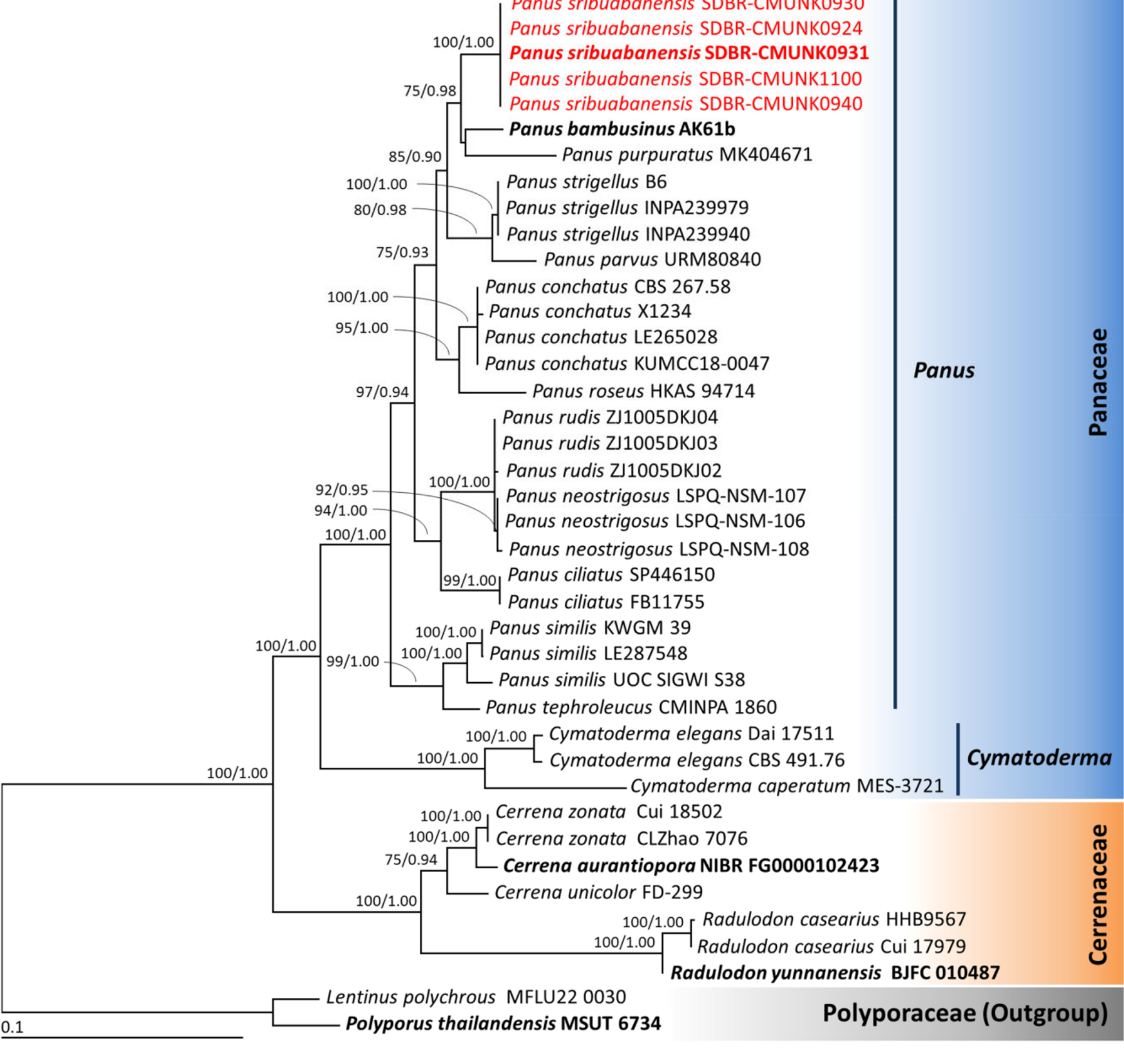

Figure 1. Phylogenetic tree derived from maximum likelihood analysis of combined ITS and nrLSU genes of 40 specimens. *Lentinus polychrous* and *Polyporus thailandensis* were set as the outgroup. Numbers above branches are the bootstrap percentages (left) and Bayesian posterior probabilities (right). Bootstrap values > 75% and Bayesian posterior probabilities > 0.90 are shown. The scale bar displays the expected number of nucleotide substitutions per site. Type species are shown in bold. Sequences derived in this study are shown in red.

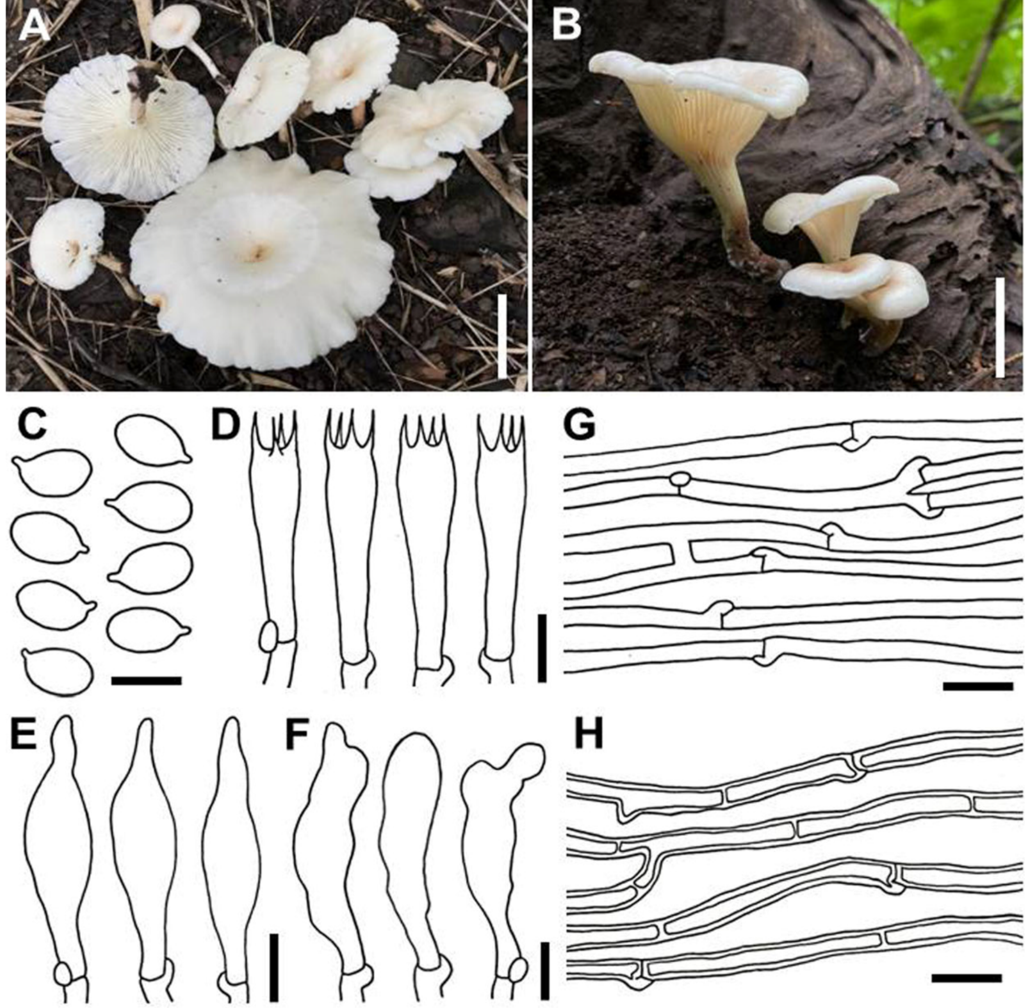

Figure 2. Basidiomata of *Panus sribuabanensis* SDBR-CMUNK0931 (holotype) (A) and SDBR-CMUNK0940 (B); microscopic features obtained from SDBR-CMUNK0931 (C–H); basidiospores (C); basidia (D); Pleurocystidia (E); cheilocystidia (F), Generative hyphae (G); Skeletal hyphae (H). Scale bars: A, B = 50 mm, C = 5 µm, D–G = 10 µm.

Description: Basidiomata medium to large. Pileus 25–160 mm diam, weakly depressed in the center or infundibuliform, concentric zone absent, squamulose when young, almost glabrous with age, brownish orange (5C4–C3) to brown (6E8) at the center when young, become orange white (5A2) to yellowish white (4A2) in age, margin entire, dentate or irregularly lobed. Hymenophore lamellate. Lamellae 3–5 tiers, decurrent, sometimes dichotomously branched, white (3A1) to yellowish white (3A2). Context up to 5 mm thick, white (3A1). Stipe 45–85 × 5–15 mm, central, cylindrical, tapering toward the base in age, solid, surface glabrous to matted fibrillose or strigose, sometimes with sparse and scattered squamules, yellowish white (4A2) near lamellae with brownish orange (5C4–C3) to brown (6E8) at the base. Odor not distinctive. Spore print white.

Basidiospores [280/5/5] (4–) 4.5–5.8 (–6) × (3–) 3.25–4 µm, L' = 5.1 µm, W' = 3.5 µm, Q = (1.30–) 1.38–1.45 (–1.50), Q_m = 1.42 ± 0.12, broadly ellipsoid to ellipsoid, hyaline, smooth, thin-walled, and inamyloid. *Basidia* 28–40 × 5.5–8.0 µm, clavate, hyaline, 4-spored, sterigmata up to 7.0 µm long. *Pleurocystidia* 32.0–70.0 × 6.5–10.0 µm, subcylindrical or fusoid-ventricose with a slightly prolonged apex, hyaline, thin-walled. *Cheilocystidia* 21.0–70.0 × 5.0–9.0 µm, versiform, generally flexuose, branched toward apex, hyaline, smooth, thin-walled, with obtuse ends. *Hyphal pegs* absent. *Hymenial trama* radially arranged and dimitic. Generative hyphae 2.0–5.5 µm wide, hyaline, smooth, thin to slightly thick-walled (up to 1 µm), frequently branched, clamp connections abundant. Skeletal hyphae dominant, 2.0–6.0 µm wide, hyaline, thick-walled (1.0–1.5 µm), mostly unbranched, rarely branched, septations not observed. *Pileal trama* radially arranged. Generative hyphae 2.0–5.0 µm wide, rarely inflated up to 10.0 µm, hyaline, smooth, thin to slightly thick-walled (up to 1 µm), frequently branched, clamp connections abundant. Skeletal hyphae dominant, 2.0–6.0 µm wide, hyaline, thick-walled (1.0–1.5 µm), mostly unbranched, rarely branched, septations not observed. *Pileipellis* cutis,

trichoderm to intricate trichoderm, 2.5–6.0 µm wide, up to 100 µm long, hyaline, thin to slightly thick-walled (up to 1.0 µm), clamp connections abundant, obtuse ends. *Stipe trama* interwoven. Generative hyphae 2.0–5.5 µm wide, hyaline, smooth, and thin to slightly thickwalled (up to 1.0 µm), frequently branched, clamp connections abundant. Skeletal hyphae 2.0–6.0 µm wide, hyaline, thick-walled (1.0–1.5 µm), mostly unbranched, rarely branched, septations not observed. *Stipitipellis* similar as pileipellis. hyphae 2–4 µm wide, hyaline, mostly thin-walled, with obtuse ends. *Clamp connections* abundant at all tissues.

Ecology and distribution: Fruiting solitary or gregarious on soil and decaying wood in a tropical deciduous forest. Known only from the type locality in northern Thailand.

Additional specimens examined: THAILAND, Lamphun Province, Mae Tha District, 18°27'41"N 99°10'30"E, elevation 427 m, 16 August 2020, J. Kumla and N. Suwannarach, SDBR-CMUNK0924 and SDBR-CMUNK0930; Mueang District, Sri Bua Ban Subdistrict, Chiang Mai University Haripunchai Campus, 18°32'26"N 99°7'31"E, elevation 475 m, on decaying wood in a tropical deciduous forest, 16 August 2020, N. Suwannarach, SDBR-CMUNK0940; 18°32'34"N 99°8'22"E, elevation 448 m, on decaying wood in a tropical deciduous forest, 10 October 2020, J. Kumla SDBR-CMUNK1100.

Note: Panus sribuabanensis was similar to *P. bambusinus*, *P. caespiticola*, and *P. tephroleucus* based on the color of the pileus. However, the longer size of the cylindrical basidiospores (6.0–8.0 × 3.0–5.5 µm) and the shorter size of basidia (18.0–20.0 × 5.0–6.0 µm) in *P. tephroleucus* differ from *P. sribuabanensis* [19,22,50]. Notably, the presence of the smaller basidiospores in *P. sribuabanensis* clearly distinguishes it from *P. caespiticola* (5.0–7.5 × 4.0–5.5 µm) [19,50]. Additionally, the narrower size of the basidiospores (4.0–6.0 × 3.0–4.0 µm, Q_m = 1.42) in *P. sribuabanensis* clearly distinguishes it from *P. bambusinus* (5.0–6.5 × 4.0–4.5 µm, Q_m = 1.32) [25,51]. Moreover, the wider size of cheilocystidia (21.0–70.0 × 5.0–9.0 µm) in *P. sribuabanensis* clearly differs from *P. bambusinus* (22.0–68.0 × 3.0–5.0 µm) [25,51]. Phylogenetically, *P. sribuabanensis* formed a monophyletic clade and sister taxon to *P. bambusinus* and *P. purpuratus* (Figure 1).

Traditionally, the main criterion for identifying *Panus* species was morphological characteristics [16,20,21,23]. On the other hand, identification could be difficult with regard to the morphological variability that can be influenced by developmental stages of basidiomata, varied environmental conditions, and geographic distributions. Thus, DNA-based techniques are essential for identification of the *Panus* species. Consequently, combined morphological characteristics and molecular data are currently used to identify *Panus* species [24–26,52]. In this study, a new edible *Panus* species discovered in northern Thailand was identified as *P. sribuabanensis* based on morphological characteristics and phylogenetic analyses. *Panus sribuabanensis* can be distinguished from certain *Panus* species with pileus colors ranging from pinkish, reddish brown, brown, to purple-gray due to its distinctive orange-white to yellowish-white pileus color. However, it shares a similar pileus color with other species like *P. bambusinus*, *P. caespiticola*, and *P. tephroleucus*, which also exhibit shades ranging from yellowish-white to pale ocher shades [19,22,50,53]. The relevant microscopic features and distribution of *P. sribuabanensis* have been compared with *P. bambusinus*, *P. caespiticola*, and *P. tephroleucus*, as is shown in Table 2. The different characteristics of the microscopic features of *P. bambusinus*, *P. caespiticola*, *P. sribuabanensis*, and *P. tephleucus* have been mentioned above. In addition, *P. bambusinus* is distributed only throughout India [25,51], while *P. caespiticola* was found in Cuba, India, Mali, Mozambique, and Tanzania [19,50]. Furthermore, *P. tephroleucus* was recorded from Brazil, Cuba, India, and Mexico [19,22,50].

According to a phylogenetic analysis of the combined ITS and nrLSU sequences, *P. sribuabanensis* established a monophyletic clade that was clearly

Table 2. Comparison microscopic features and distribution of *Panus sribuabanensis* with *P. bambusinus*, *P. caespiticola*, and *P. tephroleucus*.

Panus species	Basidiospore size (µm)	Basidia size (µm)	Cheilocystidia size (µm)	Distribution
P. bambusinus[a,b]	5.0–6.5 × 4.0–4.5	20.0–37.0 × 5.0–7.0	22.0–68.0 × 3.0–5.0	India
P. caespiticola[c,d,e]	5.0–7.5 × 4.0–5.5	25.0–35.0 × 5.0–7.0	17.0–28.0 × 5.0–8.0	Cuba, India, Mali, Mozambique, and Tanzania
P. sribuabanensis[f]	4.0–6.0 × 3.0–4.0	28.0–40.0 × 5.5–8.0	21.0–70.0 × 5.0–9.0	Thailand
P. tephroleucus[c,d,e]	6.0–8.0 × 3.0–5.5	18.0–20.0 × 5.0–6.0	14.0–25.0 × 2.0–3.5	Brazil, Cuba, India, and Mexico

[a]Vinjusha and Kumar [25].
[b]Kumar and Manimohan [51].
[c]Senthilarasu [19].
[d]Drechsler-Santos [50].
[e]Pegler [22].
[f]This study.

distinct from the other previously known *Panus* species and formed a sister taxon to *P. bambusinus* and *P. purpuratus* (Figure 1). Moreover, the phylogenetic tree clearly separated *P. sribuabanensis* from *P. bambusinus* and *P. tephroleucus*. However, there is still a need to acquire the molecular data of *P. caespiticola* to confirm its phylogenetic placement. Subsequently, a nucleotide comparison of the ITS gene indicated that *P. sribuabanensis* differs from *P. bambusinus*, *P. purpuratus*, and *P. tephroleucus* by 3.76% (20/531 bp), 7.21% (43/596 bp), and 12.16% (72/592 bp), respectively. According to Jeewon and Hyde [54], a nucleotide comparison of reliable genes must reveal a difference of more than 1.5% to confirm the existence of a new species. Therefore, *P. sribuabanensis* can be considered a new species.

3.3. Nutritional analysis

A total of three specimens of *P. sribuabanensis* were analyzed for their nutritional compositions. Specimens SDBR-CMUNK0924 and SDBR-CMUNK0930 were obtained from a roadside market and specimen SDBR-CMUNK1100 was collected from a natural forest. The results of a study involving their nutritional compositions are shown in Table 3. The analysis revealed that there were no significant differences among the specimens in relation to their levels of ash, carbohydrates, fats, dietary fiber, and protein. The contents of ash, carbohydrate, fat, fiber, and protein of *P. sribuabanensis* ranged from 6.96–7.08, 43.97–44.10, 2.58–2.67, 13.21–13.48, and 27.10–27.98% dry weight, respectively. The results indicate that this mushroom is high in carbohydrates and protein and low in fat. In this investigation, the nutritional composition of *P. sribuabanensis* was within the range previously documented in studies involving various edible mushrooms (*Agaricus bisporus*, *Auricularia* spp., *Flammulina velutipes, Lentinula edodes*, *Lentinus* spp., *Panus* spp., *Pleurotus* spp., and *Volvariella volvacea*), including ash (6.7–27.6% dry weight), carbohydrate (22.2–65.1% dry weight), fat (0.4–9.5% dry weight), fiber (3.1–14.7% dry weight), and protein (11.0–45.7% dry weight) [55–62]. According to the findings of this study, *P. sribuabanensis* can be regarded as an edible mushroom for new human food resources. In comparison to findings from previous published studies on edible *Panus* species, it was observed that the protein content of *P. sribuabanensis* was relatively higher than *P. lecomtei* (17.3%–20.0% dry weight) [29,60,61]. Additionally, *P. lecomtei* had higher carbohydrate content (44.4%–52.1% dry weight) than *P. sribuabanensis* [29,60]. The fat content in *P. sribuabanensis* was higher than that of *P. lecomtei* (0.5%–1.0% dry weight) [29,61].

3.4. Total phenolic content

The total phenolic contents of three specimens of *P. sribuabanensis* were measured and are shown in Table 4. The obtained total phenolic contents ranged from 0.66 to 0.74 mg GAE/g dw. The specimen SDBR-CMUNK0930 exhibited the highest total phenolic content, followed by SDBR-CMUNK1100. Conversely, the lowest total phenolic content was observed in specimen SDBR-CMUNK0924. According to our findings, the levels of total phenolic contents obtained from *P. sribuabanensis* were within the range of 0.39–38.44 mg GAE/g dw, which was also previously reported in edible mushrooms [63–66]. Notably, several previous studies revealed that the phenolic contents of edible mushrooms varied among different mushroom species, along with the extractability of the various solvents used in the preparation process [46,66–69]. It was found that the total phenolic content of *P. sribuabanensis* obtained in this study was lower than that of *P. conchatus* (2.73 mg GAE/g dw) [66] and *P. lecomtei* (1.70–7.80 mg GAE/g dw) [61,66]. Several previous studies found that the main phenolic compounds in the extracts of edible wild mushrooms included catechin, cinnamic acid, *p*-coumaric acid, gallic acid, hydroxycinnamic acid,

Table 4. Total phenolic content and antioxidant activity of sample extracts of *Panus sribuabanensis* in this study.

Specimen Voucher SDBR	Total phenolic content (mg GAE/g dw)	Antioxidant activity: ABTS assay (mg TE/g dw)	Antioxidant activity: DPPH assay (mg TE/g dw)	Antioxidant activity: FRAP assay (mg TE/g dw)
CMUNK0924	0.66 ± 0.05 b	0.44 ± 0.04 a	0.90 ± 0.02 b	0.93 ± 0.03 b
CMUNK0930	0.74 ± 0.04 a	0.51 ± 0.06 a	1.08 ± 0.13 a	1.08 ± 0.06 a
CMUNK1100	0.71 ± 0.04 ab	0.48 ± 0.07 a	1.01 ± 0.06 ab	1.01 ± 0.03 ab

Results are expressed as mean ± standard deviation. According to Tukey's test ($p < 0.05$), distinct letters within the same column are regarded as statistically different.

Table 3. Nutritional value on a dry basis of different specimens of *Panus sribuabanensis* in this study.

Specimen voucher SDBR	Nutritional value (% dry weight): Ash	Carbohydrate	Fat	Fiber	Protein
CMUNK0924	6.97 ± 0.15 a	43.97 ± 0.16 a	2.67 ± 0.03 a	13.21 ± 0.09 a	27.10 ± 0.26 a
CMUNK0930	7.08 ± 0.08 a	44.10 ± 0.72 a	2.58 ± 0.10 a	13.29 ± 0.22 a	27.31 ± 0.53 a
CMUNK1100	6.96 ± 0.24 a	44.07 ± 0.76 a	2.64 ± 0.11 a	13.48 ± 0.52 a	27.98 ± 0.54 a

Results are expressed as mean ± standard deviation. According to Tukey's test ($p < 0.05$), distinct letters within the same column are regarded as statistically different.

protocatechuic acid, quercetin, rosmarinic acid, and syringic acid [46,70,71]. Kakoti et al. [61] found that the main phenolic compound in extract of *P. lecomtei* is protocatechuic acid, followed by *p*-coumaric acid and cinnamic acid. This extract also contains caffeic acid, gallic acid, and vanillic acid. In this study, a spectroscopic method was used to determine the total phenol content. Therefore, the phenolic components in mushroom extracts should be further characterized and identified using additional methods, such as high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS).

3.5. Antioxidant assay

Several previous studies have found that dietary components in edible wild mushrooms serve as additional sources of natural antioxidants [46,72,73]. In this present study, the antioxidant activity of the ethanolic extracts of *P. sribuabanensis* was assessed using ABTS, DPPH, and FRAP assays. The findings indicate that the mushroom extracts exhibited positive antioxidant activities in all three methods. The resulting ABTS, DPPH, and FRAP values are shown in Table 4. It was found that the ABTS values of the three mushroom extracts were not significantly different. However, the extract from specimen SDBR-CMUNK0930 demonstrated the highest values of the DPPH and FRAP activities, followed by SDBR-CMUNK1100 and SDBR-CMUNK0924 (Table 4). These findings are in accordance with previous studies reporting that the extracts of edible mushrooms (*Agaricus* spp., *Agrocybe aegerita*, *Auricularia* spp., *Flammulina velutipes*, *Hericium erinaceus*, *L. edodes*, *Lentinus* spp., *Panus* spp., *Pleurotus* spp., *Termitomyces* spp., and *V. volvacea*) exhibited antioxidant activities [61,64,66,73–75]. Additionally, antioxidant activity of the mushroom extracts varied according to the mushroom species [46,73,75]. In this study, the ethanolic extract of *P. sribuabanensis* specimen CMUNK0930 exhibited the highest degree of antioxidant activity due to its high total phenolic content. This finding was supported by those of previous studies that demonstrated that the high levels of phenolic content are responsible for the high antioxidant activity [11,46,69,71,76]. According to Pearson correlation ($p < 0.05$), the total phenolic content of the ethanolic extracts of *P. sribuabanensis* showed a significantly strong positive correlation with ABTS ($r = 0.998$, $p = 0.03$) and DPPH ($r = 0.999$, $p = 0.01$) activities. Whereas, the positive correlation between the total phenolic content and FRAP activity ($r = 0.823$, $p = 0.38$) was not determined to be statistically significant. Previously, the antioxidant activities of the methanolic and ethanolic extracts of *P. conchatus*, *P. lecomtei*, and *P. rudis* had been reported [61,66,77]. However, their reported results make it difficult to compare the outcomes of the current research with those of previous investigations.

4. Conclusions

A new edible mushroom, *P. sribuabanensis* was discovered in northern Thailand and proposed based on a combination of morphological characteristics and phylogenetic analyses.

This mushroom was found to be high in protein and carbohydrates but low in fat content. Moreover, the ethanolic extract of this mushroom contained phenolic compounds and exhibited antioxidant activity. The results of this investigation will offer valuable knowledge on the nutritional value, total phenolic content, and antioxidant potential of this mushroom. Therefore, our results suggest that *P. sribuabanensis* has certain health benefits and can be representative of an alternative source of food and natural antioxidants for humans. Furthermore, this finding has increased the number of *Panus* species found in Thailand to seven species. Finally, the results of this study are an important step in stimulating further research on wild edible mushrooms in Thailand and may help researchers better understand the distribution of *Panus* in Asia and around the world.

Acknowledgements

The authors are grateful to Mr. Russell Kirk Hollis for kind help in the English correction.

Disclosure statement

The authors declare no competing or financial interests.

Funding

The authors gratefully acknowledge the financial support provided from Plant Genetic Conservation Project under the Royal initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (grant no. R000032658), The National Research Council of Thailand (grant no. N42A650198), and partially supported by Chiang Mai University, Thailand.

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