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Sirindhornea siamensis, A New Sequestrate Boletoid Genus of Boletaceae from Thailand

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ABSTRACT.– This study investigates sequestrate boletes from southern Thailand and presents a morphologically distinctive specimen with a strong cantaloupe-like odor. Detailed morphological and molecular phylogenetic analyses, utilizing the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU) and RNA polymerase II second largest subunit (RPB2) genes were performed to determine its taxonomic position. Nonetheless, the new sequestrate bolete exhibited a distinctive morphological features, including a scaly peridium, a characteristic cantaloupe-like odor at maturity. Together with molecular evidence, these characteristics clearly distinguish it from other sequestrate boletoid (*Chamonixia*, *Durianella*, *Octaviania*, *Rossbeevera*, and *Turmalinea*). These findings support the recognition of a novel genus within the subfamily *Leccinoideae*. Therefore, we propose a new genus and new species, *Sirindhornea siamensis*, for this sequestrate bolete species.

KEYWORDS: fungal taxonomy, new genus, Leccinoideae, sequestrate boletes, *Sirindhornea*

INTRODUCTION

The subfamily Leccinoideae within the family Boletaceae comprised a diverse group of ectomycorrhizal fungi that play crucial roles in forest ecosystems (Águeda et al., 2008; Mortimer et al., 2012). Members of subfamily Leccinoideae are known for their symbiotic associations with various tree families, including Pinaceae, Taxodiaceae, Betulaceae, Fagaceae, Fabaceae, Dipterocarpaceae and Myrtaceae (Mortimer et al., 2012). Morphologically, this subfamily encompasses both stipitate-pileate and sequestrate forms. The stipitate-pileate genera including *Leccinum*, *Leccinellum*, and *Spongispora* were characterized by a distinct cap and central stipe, typical of many bolete mushrooms (Gao et al., 2024; Wu et al., 2017; Xue et al., 2019). In contrast, the sequestrate genera such as *Chamonixia*, *Octaviania*, *Rossbeevera*, *Spongiforma* and *Turmalinea* exhibit enclosed, often hypogeous fruiting bodies that have evolved in response to dry or animal-dispersed environments (Desjardin et al., 2009; Lebel et al., 2012; Orihara et al., 2012; Orihara et al., 2016a; Orihara et al., 2016b). These divergent morphologies reflect ecological adaptations and evolutionary processes within the group. Recent molecular phylogenetic studies have significantly advanced our understanding of *Leccinoideae*, leading to the recognition of these distinct morphological lineages and the discovery of new genera and species, particularly in tropical Asia (Nuhn et al., 2013; Zeng et al., 2016; Wu et al., 2016).

Recent taxonomic and phylogenetic studies in tropical Asia have revealed an unexpected diversity of fungi within the subfamily *Leccinoideae*. Several remarkable genera have been described from Southeast Asia. *Spongiforma thailandica*, discovered in Khao Yai National Park, Thailand, represents a highly distinctive taxon with rubbery, sponge-like basidiomata and a pungent coal-tar odor. Phylogenetic analyses place it within Boletaceae, closely related to *Porphyrellus* and *Strobilomyces* (Desjardin et al., 2009). Similarly, *Spongispora temasekensis* from Singapore was described as a novel genus and species based on its unique morphology and molecular distinctness. Its globose, sponge-like basidiomata and distinct phylogenetic position further highlight the morphological diversity within *Leccinoideae* (Wu et al., 2017). *Hydnangium echinulatum*, first recorded from Malaysia, has been re-evaluated and recognized as *Durianella*, a new gasteroid bolete genus in the Boletineae, distinguished by its durian-like basidiome, Columella with indigo coloration upon exposure, and spiny basidiospores (Desjardin et al., 2008). Furthermore, *Rhodactina rostratispora*, a new sequestrate boletioid species from northern Thailand, is characterized by basidiospores with a distinct hilar appendage and terminal hilum. Phylogenetic analyses place this species in a distinct clade sister to *Spongiforma* and *Borofutus* within the *Leccinoideae* (Vadthanarat et al., 2018).

In this study, we investigated sequestrate boletes from southern Thailand and discovered a morphologically distinctive specimen emitting a strong cantaloupe-like odor. Comprehensive morphological, anatomical, and molecular phylogenetic analyses based on the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU) and RNA polymerase II second largest subunit (RPB2) genes were performed to determine its taxonomic position. While the specimen shares some general features with previously known sequestrate genera such as *Chamonixia*, *Durianella, Octaviania*, *Rossbeevera*, and *Turmalinea*, distinct morphological traits, a scaly peridium, a characteristic cantaloupe-like odor at maturity. These results support the recognition of a novel genus lineage within the subfamily *Leccinoideae*. This study aimed to describe and illustrate this new genus, and to clarify its phylogenetic position within subfamily *Leccinoideae*. Therefore, we proposed the name *Sirindhornea* for this novel genus.

MATERIALS AND METHODS

Fresh mushroom specimens were collected in February 2021 from the Sai Rung Waterfall Forest Preservation Unit, Khao Banthat Wildlife Sanctuary, Yan Ta Khao District, Trang Province, and Phatthalung Wildlife Breeding Center in Khao Banthat Wildlife Sanctuary, Srinagarindra District, Phatthalung Province, Thailand (Fig. 1). The collection localities of the specimens were listed in Table 1.

Photographs of the fresh specimens were captured from various angles in their natural habitats using a Fuji XA-5 camera for further analysis. Observations regarding potential host plants and the surrounding environments where the fruiting bodies were found were also documented. The fresh basidiocarps were carefully transported to the laboratory in paper bags. Macroscopic features—including color, size, shape, surface texture of the fruiting bodies, and host substrates—were recorded to aid in taxonomic identification. Specimens were dried in closed containers containing activated silica gel and subsequently deposited in the BIOTEC Bangkok Herbarium (BBH).

Genomic DNA was extracted using the Favor Prep^{TM} Genomic DNA Extraction Kit. The internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU) and RNA polymerase II second largest subunit (RPB2) regions were amplified using the primer pairs ITS1/LR7 (White et al. 1990; Vilgalys and Hester 1990) and 5F/7CR (Liu et al., 2000), respectively PCR reactions were carried out in a total volume of 25 µL, containing 1× Thermoscientific DreamTaq^{TM} Green PCR Master mix and 0.5 µM of each primer and 2 µL of genomic DNA tempalte. Amplification conditions followed the protocol outlined by Leetanasaksakul et al. (2024). Sequencing was performed using six primers: ITS1, ITS4, LR0R, LR3, LR5, and LR7 for the ITS–LSU region, and 5F and 7CR for the RPB2 gene. The resulting sequence fragments were assembled into contiguous ITS–LSU and RPB2 regions and subsequently submitted to GenBank (Table 1).

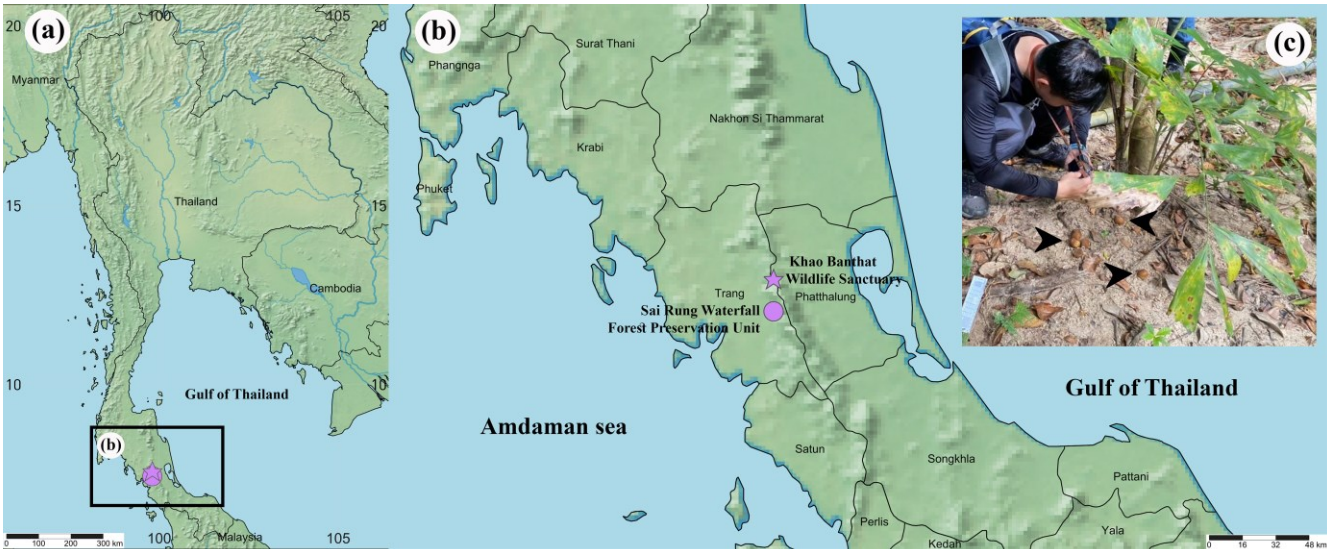

FIGURE 1. A. Map of Thailand showing the location where *Sirindhornea siamensis* was discovered in southern Thailand. **B.** The locations of both sample sites: hatthalung Wildlife Breeding Center, Khao Banthat Wildlife Sanctuary, Srinagarindra District, Phatthalung, Thailand (purple star) and Sai Rung Waterfall Forest Preservation Unit, Khao Banthat Wildlife Sanctuary, Yan Ta Khao District, Trang, Thailand (purple circle). **C.** The sequestrate basidiomata of *Sirindhornea siamensis* on sandy loam soil under the Burmese fishtail palm (*Caryota urens*).

TABLE 1. Properties of new sequestrate bolete specimens examined in this study.

Strain	Source	Locality	GenBank Accession No.*		Sample collection date
			ITS&LSU	RPB2	
AGW06 = BBH 50084^T	Basidiomata on sandy loam soil under the Burmese fishtail palm (*Caryota urens*)	Phatthalung Wildlife Breeding Center, Khao Banthat Wildlife Sanctuary, Srinagarindra District, Phatthalung, Thailand (7°34'48.0"N 99°48'36.0"E)	LC661382	LC895541	6/2/2021
SR05 = BBH 50082	Basidiomata on sandy loam soil under the Burmese fishtail palm (*Caryota urens*)	Sai Rung Waterfall Forest Preservation Unit, Khao Banthat Wildlife Sanctuary, Yan Ta Khao District, Trang, Thailand (7°26'24.0"N 99°48'36.0"E)	LC661383	LC895542	7/2/2021

*Gene sequences: ITS, internal transcribed spacer regions; LSU, the nuclear large subunit rRNA gene, RNA polymerase II second largest subunit; RPB2

Individual analyses were conducted for the ITS, LSU and RPB2 datasets, each comprising 42 sequences representing members of the subfamily Leccinoideae. Raw DNA sequences were assembled using BioEdit v.7.0.5.3 (Hall, 1999) and subsequently deposited in GenBank, with accession numbers provided in Table 1. The dataset of taxa within the subfamily Leccinoideae followed Orihara et al. (2021) and was retrieved from GenBank for use in phylogenetic analyses (Supplementary 1). Sequence alignments were conducted using MEGA X. Phylogenetic trees based on the concatenated ITS, LSU, and RPB2 datasets were generated using the Maximum Likelihood (ML) method with the General Time Reversible (GTR) model implemented in MEGA X, and branch support was evaluated with 1,000 bootstrap replicates.

For microscopy hand-cut sections of fresh or dried specimens were mounted in 3% KOH. To determine the amyloid reaction, dried material was stained with melzer's reagent. Basidiospore dimensions (e.g., range of spore size, length of appendages) and their standard deviations (SD) were determined based on 50 measurements. Basidiospore morphology was investigated using a scanning electron microscope (SEM) based on the procedure described by Haranto et al. (2024). For SEM sample preparation, specimens were initially fixed overnight in 2% glutaraldehyde prepared in a 50 mM phosphate buffer (pH 7.2). They were then dehydrated through a graded ethanol series, increasing by 10% increments from 10% to 100%, with each step lasting one hour. Following dehydration, samples were subjected to critical point drying and subsequently sputter-coated with gold. Imaging was performed using a Quanta 450 SEM (FEI Technologies Inc., Hillsboro, OR, USA).

For anatomical observation, the paraffin technique was conducted following Ruzin (1999). The fresh basidiocarps were fixed in FAAII (formaldehyde: glacial acetic acid: 50% ethanol, 5:5:90 v/v/v) for 48 hours, then washed three times with 50% ethanol for three hours each. The samples were then dehydrated through a graded series of tert-butyl-ethyl alcohol (50%, 70%, 85%, 95%, and 100%) (Suthinon et.al., 2021), followed by infiltration and embedding in Histoplast paraffin wax (Leica Biosystems, Singapore). The embedded samples were sectioned into 15 µm thick slices using a rotary microtome (Leica, Germany). Sections were stained with Safranin and Fast Green, and images were captured using a light microscope (Nikon Instech Co., Ltd., Tokyo, Japan). Additionally, taxonomic information for the fungal specimens was submitted to MycoBank.

RESULTS AND DISCUSSIONS

Molecular phylogeny

Two sequences were newly generated in this study (Table 1). Phylogenetic analysis was conducted using a combined ITS, LSU and RPB2 dataset comprising 42 boletoid fungi representing thirteen genera within the subfamily *Leccinoideae*, including, *Borofutus*, *Chamonixia*, *Durianella Leccinum*, *Leccinellum*, *Garcileccinum*, *Octaviania*, *Spongiforma, Spongispora*, *Retiboletus, Rossbeevera, Turmalinea* and *Tylocinum*. An additional Boletaceae species, *Strobilomyces brunneolepidotus*, was used as the outgroup (Fig. 2). Individual phylogenetic analyses of ITS, LSU, and RPB2 are provided in Supplementary 2.

The analyses strongly support the placement of the two specimens as a distinct lineage, representing a new genus separate from other sequestrate genera within *Leccinoideae*, including *Chamonixia*, *Durianella*, *Octaviania*, *Rossbeevera*, and *Turmalinea* as well as from the boletoid genera *Leccinum* and *Leccinellum*. (Fig. 2). Distinct morphological features, particularly the scaly peridium that cracks at maturity (Fig. 3A), further distinguish this lineage. Considering both molecular and morphological evidence, the new genus *Sirindhornea* is introduced to accommodate this lineage.

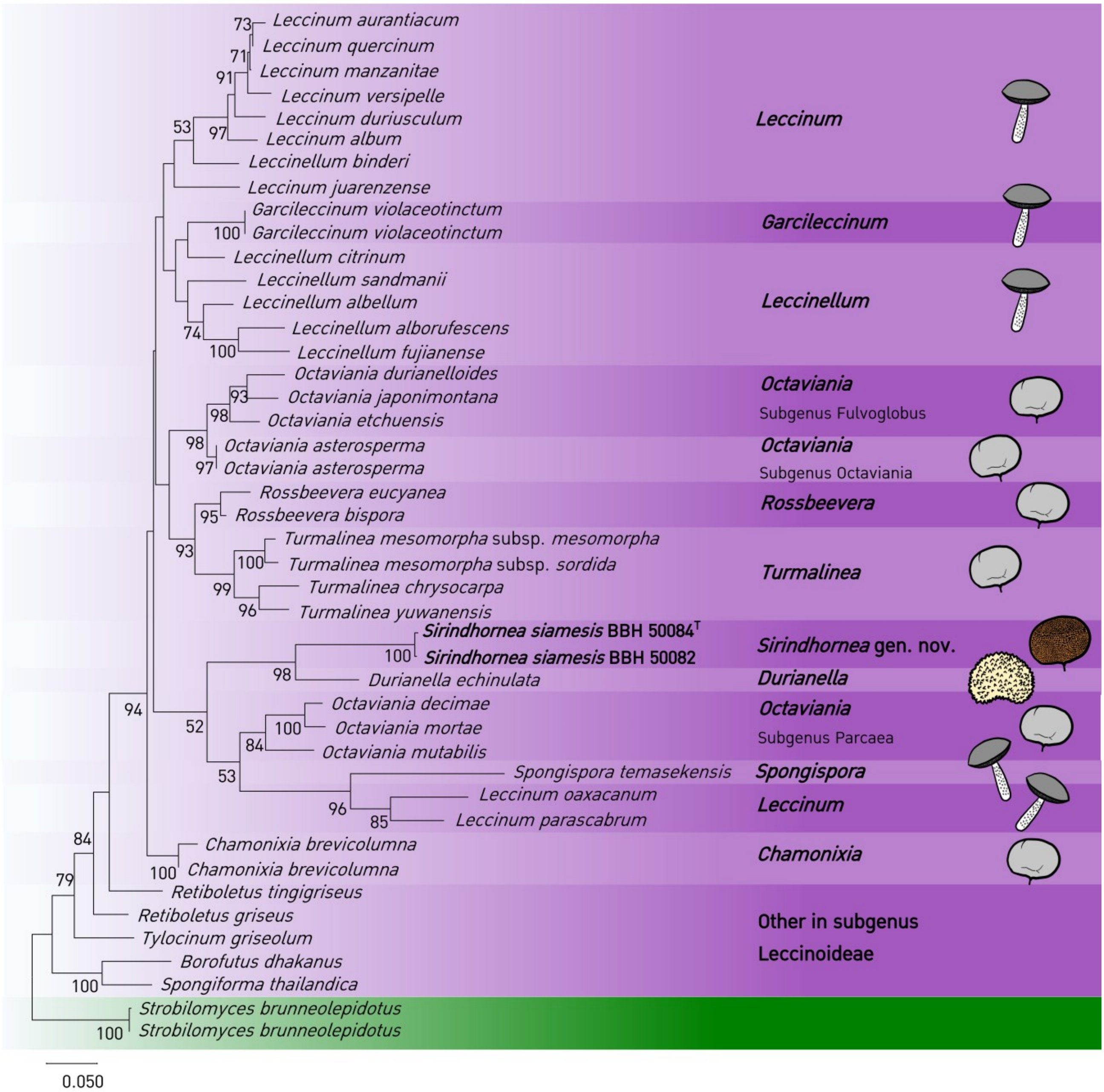

FIGURE 2. Maximum likelihood (ML) phylogenetic tree of fungi in the subfamily *Leccinoideae*, constructed from a concatenated dataset of ITS, LSU rRNA and RPB2 gene sequences, illustrating the phylogenetic placement of the novel genus *Sirindhornea* relative to closely related taxa. Bootstrap support values ≥50% are shown at the nodes. *Strobilomyces brunneolepidotus* was used as the outgroup. The scale bar represents a patristic distance of 0.050 substitutions per site. The images on the right indicates whether the genus belongs to the stipitate-pileate or sequestrate form.

Taxonomy

Sirindhornea gen. nov.

Mycobank MB 858881

Etymology.– The genus name Sirindhornea (si.rin.dhorn.ne.a) is named in honour of Her Royal Highness Maha Chakri Sirindhron on the occasion of her 70th birthday, in recognition of her interest in natural history and her contributions to biodiversity conservation and environmental protection in Thailand.

Type species.– *Sirindhornea siamensis* gen. and sp. nov.

Diagnosis.– This genus is distinguished from all other members of the Boletaceae by a unique combination of molecular, and morphological features. The peridium is yellowish-brown with a scaly surface that becomes cracked and fragmented at maturity, in contrast to the smooth peridial surfaces observed in *Chamonixia*, *Octaviania*, *Rossbeevera*, and *Turmalinea*, while *Durianella*

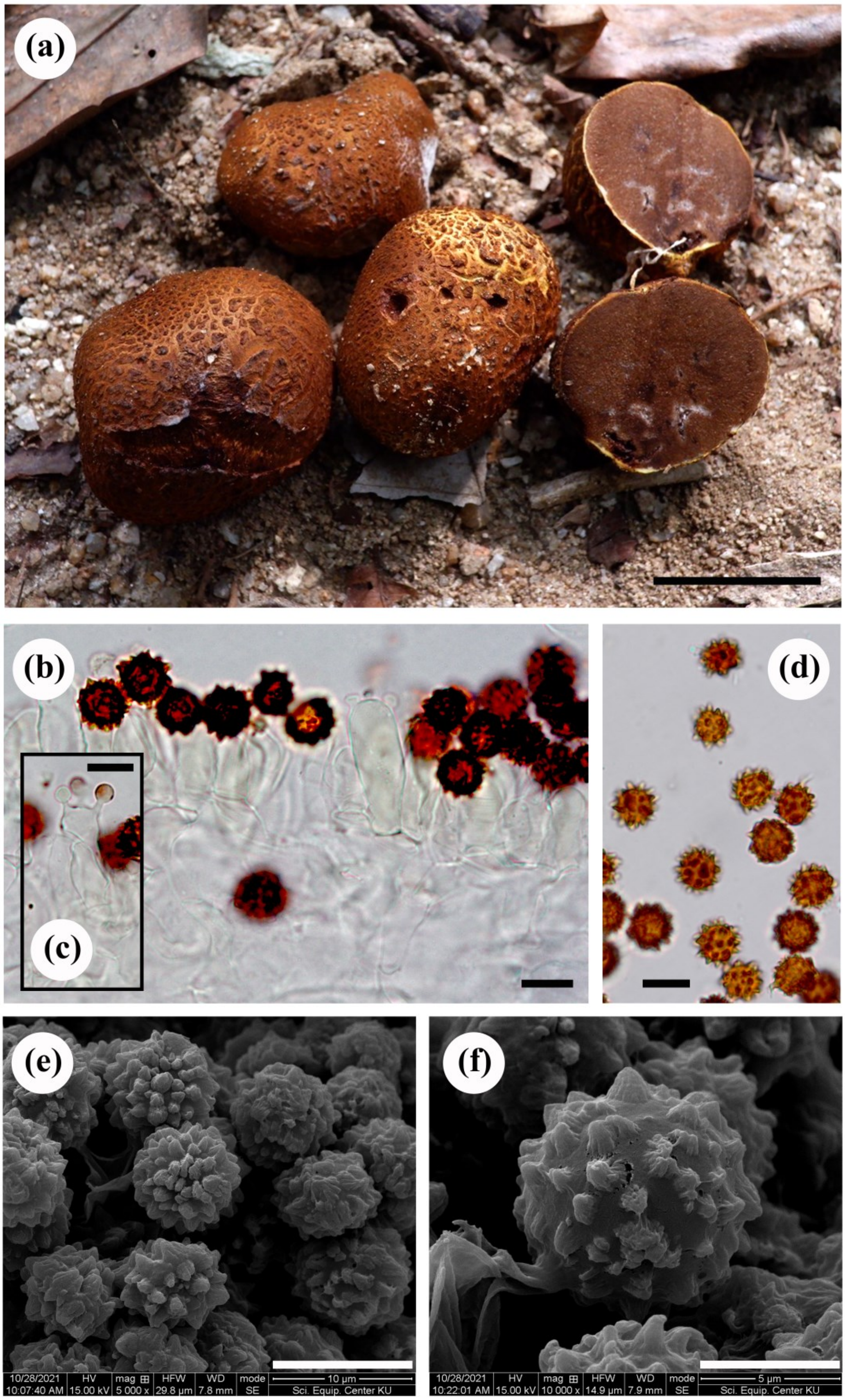

FIGURE 3. *Sirindhornea siamensis* (BBH 50084, holotype). **A.** Basidiomata. **B.** Basidia with mature basidiospores mounted in 3% KOH. **C.** Basidium with developing basidiospores. **D.** Basidiospores under light microscopy, golden brown with coarse spines to tubercles ornamentation. **E**, **F.** Basidiospores under SEM. Scale bars: **A** = 3 cm., **B**–**E** = 10 µm. and (f) = 5 µm.

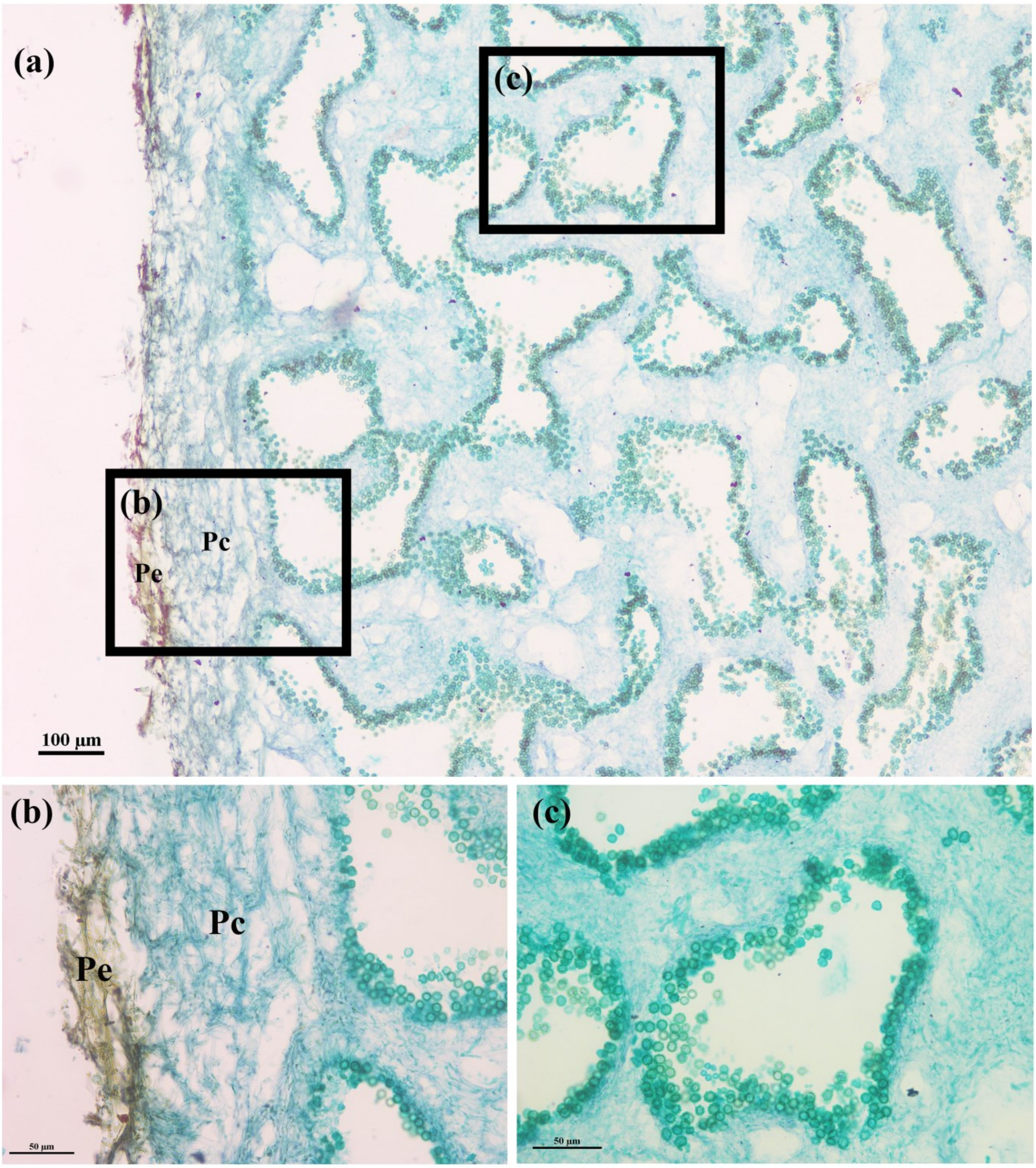

FIGURE 4. Microstructures of *Sirindhornea siamensis* (BBH 50084, holotype). **A.** Longitudinal section of the basidioma, showing the internal hymenophoral trama and enclosing peridium. **B.** Peridiopellis and peridial context composed of loosely interwoven hyphae. (c) Hymenophoral trama with a poroid structure, containing basidia and basidiospores. Scale bars: **A** = 100 µm., **B, C** = 50 µm. Pe= Peridiopellis and Pc = peridial context

has pyramidal-warted, durian fruit-like basidiomata. It can be further differentiated from other genera by basidiospore morphology: *Rossbeevera* and *Turmalinea* produce ellipsoid to fusoid basidiospores (Lebel et al., 2012; Orihara et al., 2017), whereas *Octaviania* (Orihara et al., 2021) and *Sirindhornea* produce globose to subglobose spores, with *Octaviania* having large, coarse, pyramidal spines (1.1–3.4 µm high) forming sharply angular, stellate projections, and *Sirindhornea* having densely packed, short, blunt tubercles less than 1 µm high, giving the spore surface a granular and clustered appearance; additionally, mature *Sirindhornea* basidiomata emit a distinctive cantaloupe-like odor, which differs from *Spongiforma*, which has a vaguely fruity or strongly musty odor (Desjardin et al., 2009).

Description.– Basidiomata sequestrate, globose to subglobose, occasionally oblong-globose, partially emergent from sandy loam soil under *Caryota urens* (Arecales). White rhizomorphs present at base. Peridium yellowish- to greenish-brown, scaly, cracking and fragmenting at maturity, enclosing solid gleba darkening to brown. Peridium thin, 0.1–0.15 mm thick.

Sirindhornea siamensis sp. nov.

Mycobank MB861120

(Figs 2–4)

Etymology.–The species epithet siamensis (si.am.men. sis) is named from the former name of Thailand, refers to the geographical origin of this new species.

Classification.– Leccinoideae, Boletaceae, Boletales, Agaricomycetes, Agaricomycotina, Basidiomycota

Holotype.– Thailand: Phatthalung Province, Srinagarindra District, Khao Banthat Wildlife Sanctuary, 6 Feb 2021, B. Sakolrak, P. Sintawarak, P. Jangsantear & T. Boonwanno (AGW06 = BBH 50084,– holotype)

Diagnosis.–*Sirindhornea siamensis* is distinguished from other sequestrate members of the Boletaceae by its yellowish-brown, scaly peridium that becomes cracked and fragmented at maturity, the presence of white rhizomorphs at the base, and the emission of a distinct cantaloupe-like odor when mature. The basidiospores are globose to subglobose, golden brown, ornamented with densely packed, short, blunt spines to tubercles less than 1 µm high, giving the surface a granular appearance. The basidia are clavate, four-spored, and cystidia are rare. Clamp connections are absent. It differs from the type species of related genera such as *Octaviania*, *Rossbeevera*, and *Turmalinea* by the combination of smaller, bluntly ornamented spores, thinner peridium, and the distinctive fruity odor.

Description.– Basidiomata sequestrate, globose to subglobose, occasionally oblong-globose when young, 1.1–3.5 cm in diameter, partially emergent from soil (Fig. 3A). White rhizomorphs present at base in both young and mature specimens. Peridium yellowish-brown, scaly, opaque, dull, cracking and fragmenting at maturity, enclosing solid gleba turning brownish to dark brown. Peridium relatively thin, 0.1–0.15 mm thick, producing cantaloupe-like odor at maturity.

Basidia clavate, smooth, thin-walled, hyaline, bearing four basidiospores arising from hymenophoral trama (Fig. 3B, C), measuring (19–)21.6–26.4(–27.7) × (7–) 8–9.8(–10.4) µm (n = 50; mean = 24 ± 2.4 × 8.9 ± 0.9 µm). Cystidia rare, measuring (22.8–)25.2–33.2(–37.5) × (7.5–)8–9.6(–10) µm (n = 15; mean = 29.2 ± 4 × 8.8 ± 0.8 µm). Clamp connections absent.

Basidiospores globose, golden brown, (7.87–)8.3–9.5(–10.5) × (5.9–)7.2–8.8(–9.2) µm (n = 50; mean = 8.9 ± 0.6 × 8.0 ± 0.8 µm), ornamentation coarse spines to tuberculate (Fig. 3D). Scanning electron micrographs show densely distributed spines on spore surface (Fig. 3E, F).

Longitudinal section of basidioma shows well-developed gleba enclosed by distinct peridium (Fig. 4A). Peridiopellis compact layer of hyphae, underlying peridial context composed of loosely arranged, thin-walled, hyaline hyphae (Fig. 4B). Hymenophoral trama poroid, basidia and mature basidiospores embedded within (Fig. 4C).

Distribution.– The sequestrate basidiomata were observed on sandy loam soil under the Burmese fishtail palm (*Caryota urens*) in tropical rainforest of southern Thailand (Fig. 1, Table 1).

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SUPPLEMENTARY 1

TABLE S1. List of fungi used in this study.

Species/Subspecies	Isolate No.	ITS	LSU	RPB2
Borofutus dhakanus	HKAS73792	JQ928607	JQ928617	JQ928598
Chamonixia brevicolumna	HAD1315	PV591200	PV591282	-
Chamonixia brevicolumna	DBG F28707	-	MK601729	MK766291
Durianella echinulata	AW-2007a	EU293061	EU293063	-
Garcileccinum violaceotinctum	CFMR BZ-3169	MN250215	MK601780	MK766338
Garcileccinum violaceotinctum	CFMR:BZ-1676	MN250203	MK601779	MK766337
Leccinellum albellum	MICH:KUO-09200807	-	MK601747	MK766309
Leccinellum alborufescens	FHMU1758	MK816326	MK816321	MK816332
Leccinellum binderi	KD 22-015	-	OQ858379	OQ914387
Leccinellum citrinum	HKAS:53427	-	KF112488	KF112727
Leccinellum fujianense	FHMU2223	MK816325	MK816320	MK816336
Leccinellum sandmanii	DP5445	PV241500	PV241501	PV259854
Leccinum album	Li1072	MZ392873	MW413907	MW439260
Leccinum aurantiacum	LJW270	MZ485405	MZ675540	MZ707778
Leccinum duriusculum	Yang5971	MZ485402	MZ675541	MZ707779
Leccinum manzanitae	NY:14041	-	MK601765	MK766324
Leccinum juarenzense	MEXU HO 30112	OQ984885	NG243288	OQ938916
Leccinum oaxacanum	MEXU HO 30460	OQ984886	NG243289	OQ971864
Leccinum parascabrum	Li1700	MZ392875	MW413912	MW439265
Leccinum quercinum	HKAS:63502		KF112444	KF112724
Leccinum versipelle	FB27	MZ485401	MZ675546	MZ707782
Octaviania durianelloides	KPM-NC 27371	MT934813	MT812733	-
Octaviania etchuensis	KPM NC-0017822	NR120080	NG042595	-
Octaviania japonimontana	KPM-NC 27623	MT934802	MT812718	-
Octaviania mutabilis	KRA F-2012-99	MT934787	MT812707	-
Octaviania asterosperma	FH-284311	-	MK601796	MK766353
Octaviania asterosperma	FH-284316	-	MK601795	MK766352
Octaviania decimae	KPM NC-0017763	NR119977	NG042592	-
Octaviania mortae	KPM-NC-0017770	JN257994	JN378470	-
Retiboletus griseus	Halling10162	OM904961	MT010608	ON004088
Retiboletus sinensis	HKAS122944	OM904987	OM984676	-
Retiboletus tingigriseus	NM-2023a	OR655158	OR655202	OR659954
Rossbeevera bispora	GDGM 45639		MK036347	MK350309
Rossbeevera eucyanea	KPM-NC 28182	MT934782	MT812702	
Sirindhornea siamensis	**BBH 50084^{T}**	**LC661382**		**LC895541**
Sirindhornea siamensis	**BBH 50082**	**LC661383**		**LC895542**
Spongiforma thailandica	DED7873	NR119699	NG042464	MG212648
Spongispora temasekensis	SING 0206334	MG979396	MG979397	MG979393
Turmalinea mesomorpha subsp. *mesomorpha*	KPM-NC-0018014	KC552000	KC552048	-
Turmalinea mesomorpha subsp. *sordida*	KPM-NC-0017743	KC552002	KC552050	-
Turmalinea chrysocarpa	HKAS70601	KC552003	KC552051	-
Turmalinea yuwanensis	KPM-NC-0018011	KC551998	KC552046	-
Tylocinum griseolum	HKAS50209	-	KT990551	KT990388
Strobilomyces brunneolepidotus	N.K.Zeng2575	MT822947	MT829135	-
Strobilomyces brunneolepidotus	HKAS81935	MG832053	-	KX869520

SUPPLEMENTARY 2

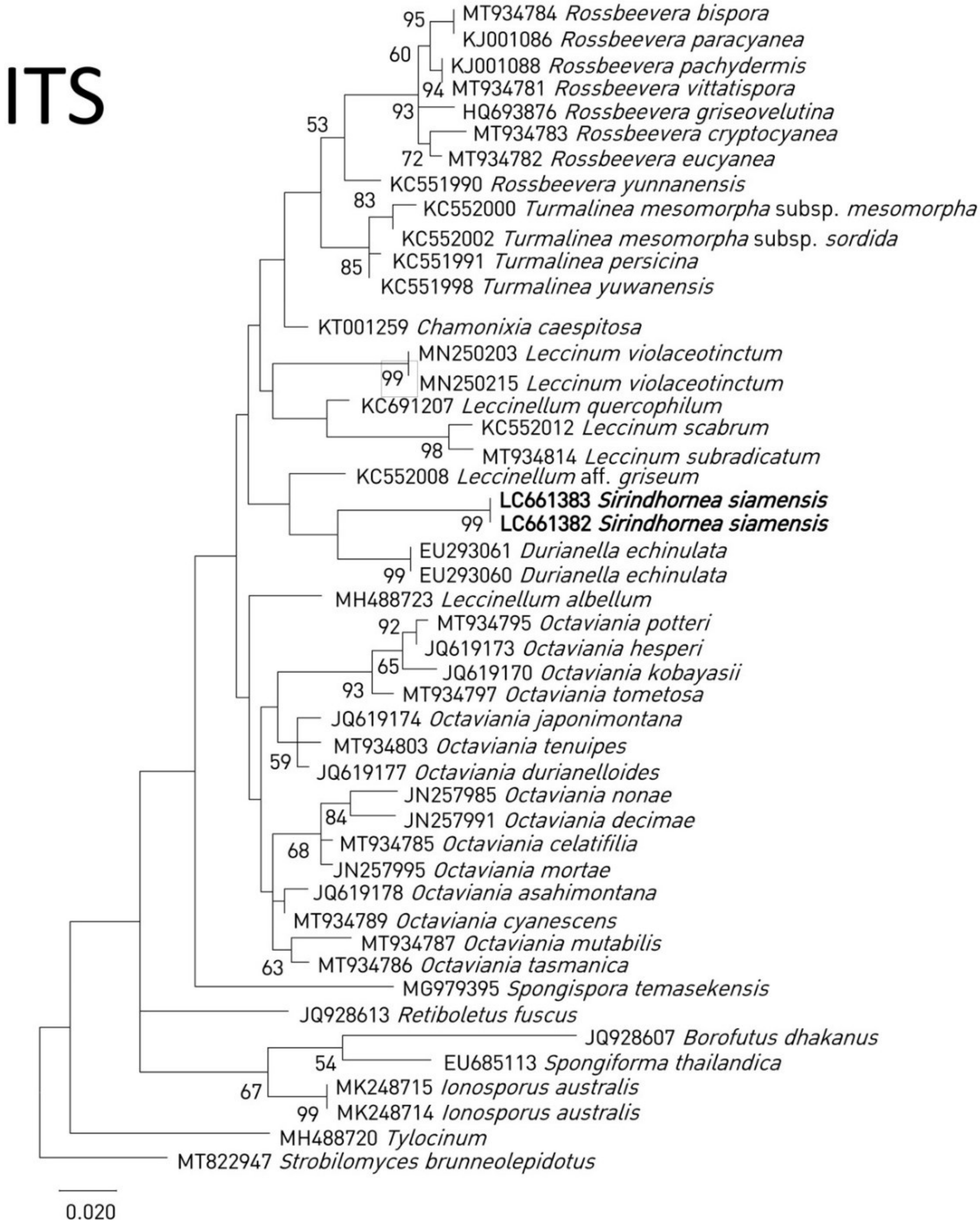

FIGURE S1-1. Maximum likelihood (ML) phylogenetic tree of fungi in the subfamily *Leccinoideae*, constructed from a concatenated dataset of ITS gene sequences, illustrating the phylogenetic placement of the novel genus *Sirindhornea* relative to closely related taxa. Bootstrap support values ≥50% are shown at the nodes. *Strobilomyces brunneolepidotus* was used as the outgroup. The scale bar represents a patristic distance of 0.020 substitutions per site.

SUPPLEMENTARY 2

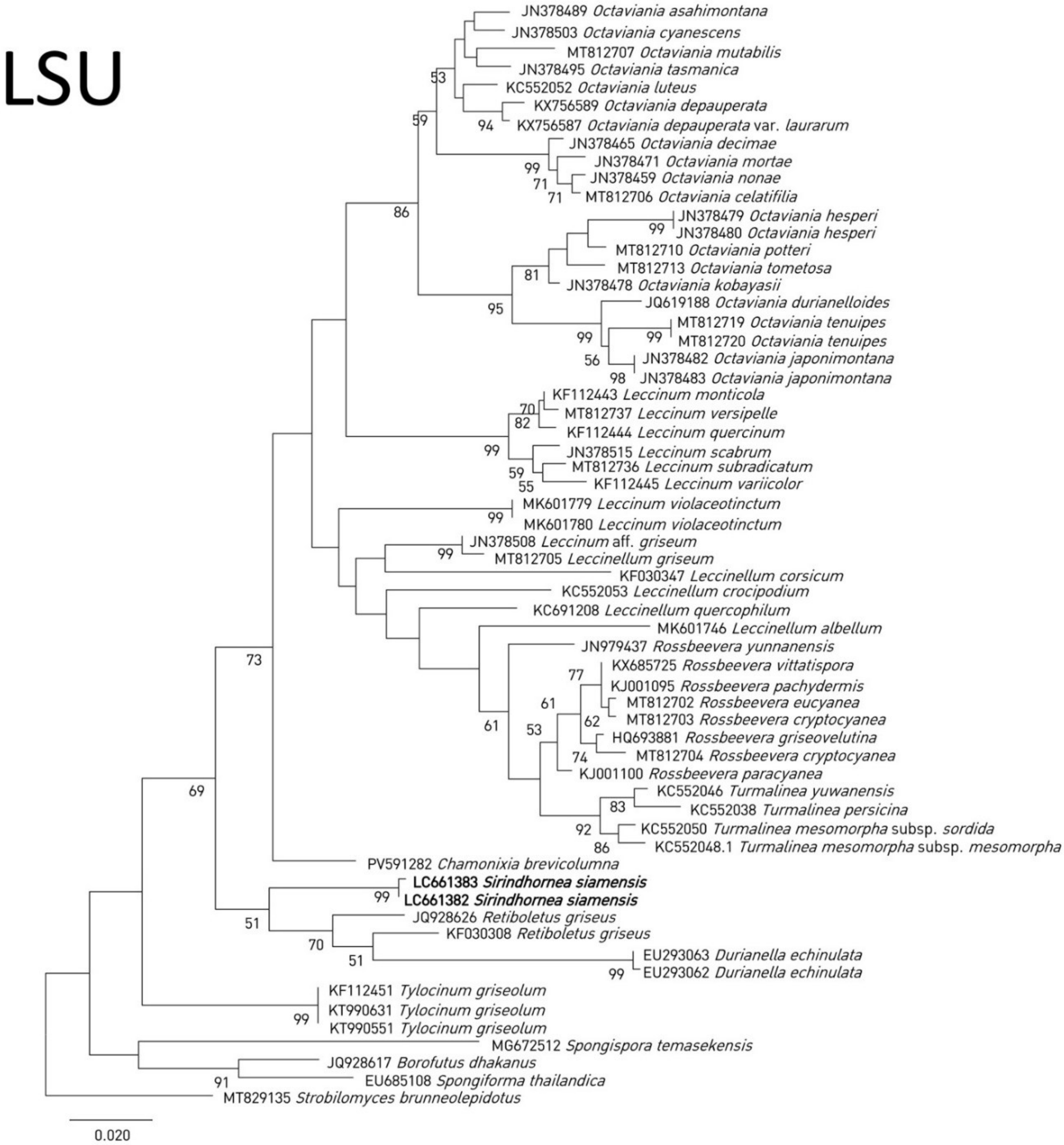

FIGURE S1-2. Maximum likelihood (ML) phylogenetic tree of fungi in the subfamily *Leccinoideae*, constructed from a concatenated dataset of LSU gene sequences, illustrating the phylogenetic placement of the novel genus *Sirindhornea* relative to closely related taxa. Bootstrap support values ≥50% are shown at the nodes. *Strobilomyces brunneolepidotus* was used as the outgroup. The scale bar represents a patristic distance of 0.020 substitutions per site.

SUPPLEMENTARY 2

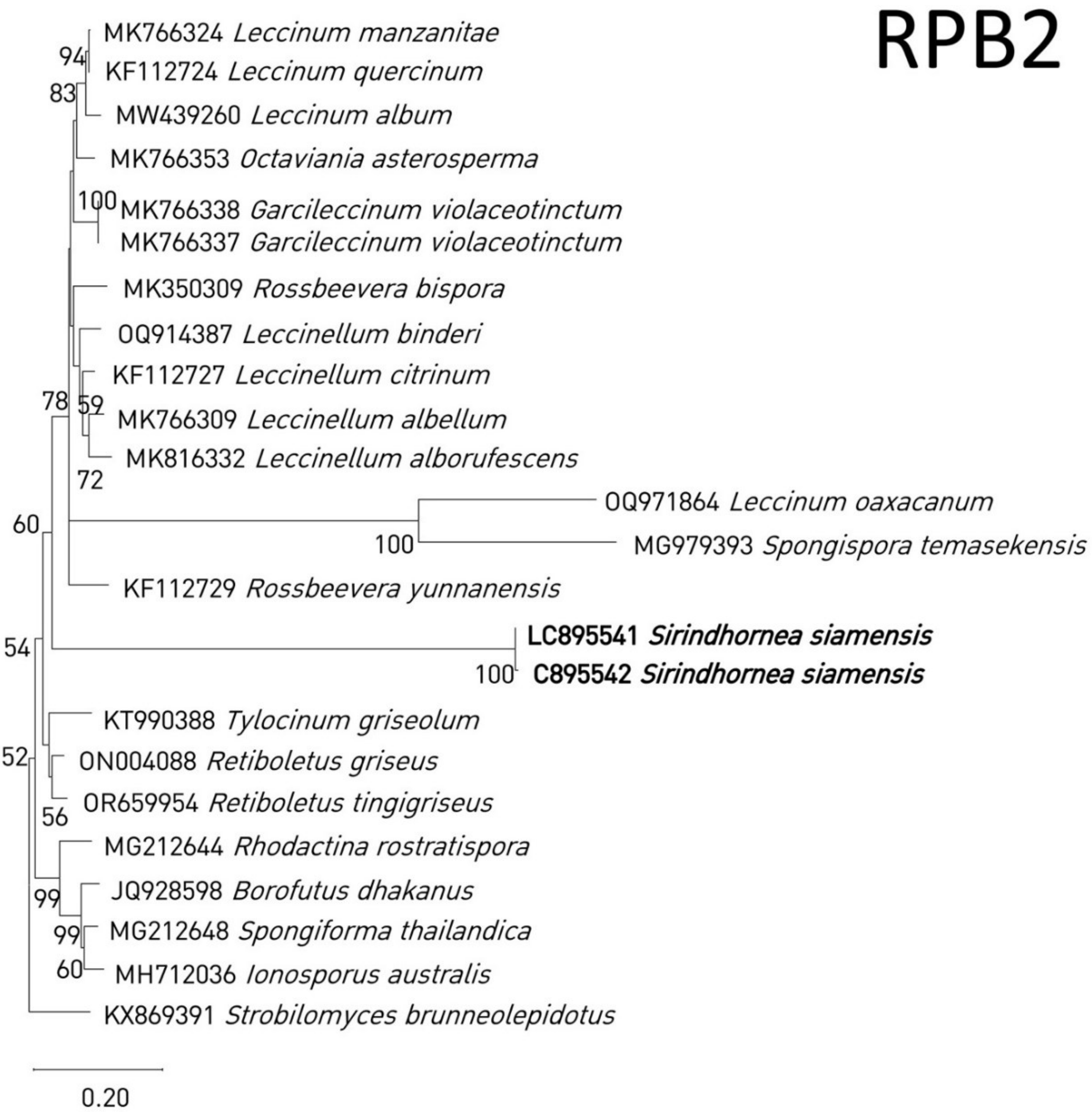

FIGURE S1-3. Maximum likelihood (ML) phylogenetic tree of fungi in the subfamily *Leccinoideae*, constructed from a concatenated dataset of RPB2 gene sequences, illustrating the phylogenetic placement of the novel genus *Sirindhornea* relative to closely related taxa. Bootstrap support values ≥50% are shown at the nodes. *Strobilomyces brunneolepidotus* was used as the outgroup. The scale bar represents a patristic distance of 0.20 substitutions per site.