

## *Calathella sirindhorniae* (Agaricales, Basidiomycota), A New Cyphelloid Fungus from Thailand

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**ABSTRACT.**— A novel cupulate basidiomycete fungus was discovered during a survey of mangrove-associated fungi in Chanthaburi and Phetchaburi Provinces, Thailand. This species, found exclusively on decaying *Rhizophora mucronata* hypocotyls in intertidal zones, forms minute fruiting bodies and shows close morphological and molecular resemblance to *Calathella mangrovei* Jones & Agerer, 1992. Phylogenetic analyses of ITS and LSU rDNA regions confirmed its placement within *Calathella*, as a distinct sister lineage to *C. mangrovei*. Therefore, these isolates represent a novel species, for which the name *Calathella sirindhorniae* sp. nov. is proposed.

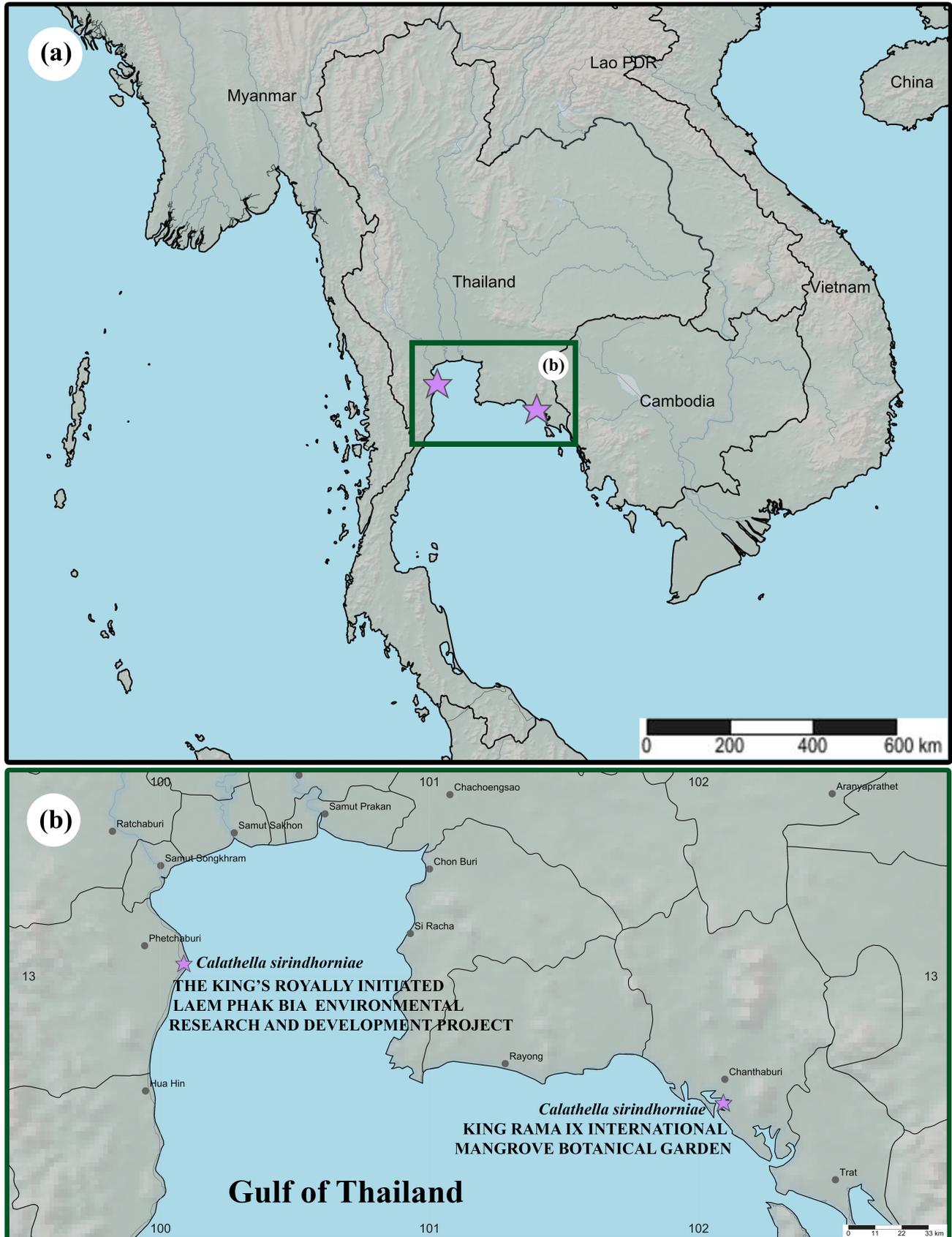
**KEYWORDS:** *Calathella sirindhorniae*, cyphelloid fungus, fungal taxonomy, new species, mangrove

### INTRODUCTION

Cyphelloid fungi are a morphologically diverse group of basidiomycetes, typically forming small, cup- or disc-shaped fruiting bodies on decaying wood or plant material in moist environments (Hibbett et al., 2014). Although once considered morphologically simple, cyphelloid fungi exhibit considerable taxonomic diversity and ecological significance, particularly in forest and mangrove ecosystems (Sulzbacher et al., 2008; Halama et al., 2019). Prominent cyphelloid genera include *Calathella*, *Lachnella*, *Cyphellopsis*, *Flagelloscypha*, *Henningsomyces*, *Merismodes*, *Eoscyphella*, *Nia*, and *Phaeosolenia* (Bodensteiner et al., 2004). The newest member of the cyphelloid genera is *Cyphelloporia bialoviesensis* (Karasiński et al., 2023). Among these, the genus *Calathella* (family Marasmiaceae, order Agaricales) comprises five species, namely *C. columbiana* (Sulzbacher et al., 2008), *C. digitiformis* (Bodensteiner et al., 2001), *C. eruciformis* (Halama et al., 2019), *C. gayana* (Agerer, 1983), and *C. mangrovei* (Jones and Agerer, 1992), which are morphologically characterised by their minute, white to cream-coloured basidiomata and smooth hymenial surfaces (Jones and Agerer, 1992). *Calathella* species are generally saprotrophic and found on decaying wood in temperate and tropical regions. However, the diversity and distribution of *Calathella* in Southeast Asia, particularly in mangrove habitats, remain poorly documented (Jones et al., 2015).

A research project on the biodiversity of microbial associated with mangrove leaf and soil sediment in the King Rama IX International Mangrove Botanical Garden, located in Mueang Chanthaburi District, Chanthaburi Province, Thailand was conducted in 2023. The primary objective of the project was to explore the diversity of microorganisms including filamentous fungi, yeasts, bacteria, and actinomycetes associated with *Rhizophora mucronata* Poir. leaf and mangrove sediments. This research was carried out as a subsidiary project under the broader framework of a multidisciplinary study focusing on microbial communities in mangrove ecosystems. During the course of the survey, a cyphelloid fungus morphologically similar to *Calathella mangrovei* Jones and Agerer (1992) was repeatedly found. Consequently, the survey area was extended ecologically similar intertidal zones along the Gulf of Thailand to locate additional specimens. In January 2025, a similar specimen was also collected from the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project in Phetchaburi Province, Thailand. Morphological observations combined with phylogenetic analyses based on nuclear ribosomal DNA sequences, specifically the internal transcribed spacer (ITS) and large subunit ribosomal RNA regions, confirmed that these specimens represent a novel species within the genus *Calathella*.

This study aims to describe and illustrate this new species and to clarify its phylogenetic position within *Calathella*, thereby contributing to the understanding of fungal diversity in the mangrove ecosystems of



**FIGURE 1.** Map of Thailand. **A.** The locations where *Calathella sirindhorniae* was discovered, indicated by a purple star. **B.** The locations of both sample sites: the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project and the King Rama IX International Mangrove Botanical Garden.

TABLE 1. Properties of new cyphelloid fungi strains examined in this study.

Strain	Source	Location	GenBank Accession No.*		Locality	Sample collection date
			ITS	LSU		
DMKU-PKL2024-001 =BBH 50085 <sup>T</sup>	Decay hypocotyl of <i>Rhizophora mucronata</i>	King Rama IX International Mangrove Botanical Garden, Mueang Chanthaburi District, Chanthaburi Thailand	LC794554	LC866789	12°31'33.42"N 102°5'33.01"E	24/12/2023
DMKU-PKL2024-002	Decay hypocotyl of <i>Rhizophora mucronata</i>	King Rama IX International Mangrove Botanical Garden, Mueang Chanthaburi District, Chanthaburi Thailand	LC794555	LC866790	12°31'31.56"N 102°5'30.32"E	25/12/2023
DMKU-PKL2024-003	Decay hypocotyl of <i>Rhizophora mucronata</i>	King Rama IX International Mangrove Botanical Garden, Mueang Chanthaburi District, Chanthaburi Thailand	LC794556	LC866791	12°31'31.02"N 102°5'32.98"E	25/12/2023
DMKU-PKL2025-001	Decay hypocotyl of <i>Rhizophora mucronata</i>	The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem district, Phetchaburi province Thailand	LC866787	LC866788	13°2'52.6"N 100°5'11.23"E	27/1/2025

\*ITS, internal transcribed spacer regions; LSU, the nuclear large subunit rDNA.

Southeast Asia. Moreover, this research represents the first record of the genus *Calathella* in Thailand, highlighting the largely unexplored fungal diversity of Thailand's mangrove habitats.

## MATERIALS AND METHODS

Fresh fungal specimens were collected from the King Rama IX International Mangrove Botanical Garden, Mueang Chanthaburi District, Chanthaburi, Thailand, and the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province, Thailand, in December 2023 and January 2025, respectively. (Fig. 1). The localities of the specimens were shown in Table 1.

Photographs of the fresh specimens were taken from multiple angles in their natural environments using a Nikon D7500 camera (Nikon Instech Co., Ltd., Tokyo, Japan) for subsequent analyses. Observations of potential host plants and the environments where the fruiting bodies were discovered were also noted. The fresh basidiocarps were carefully wrapped in moist newspaper, placed in a humid chamber, and transported to the laboratory. Macroscopic characteristics, including colour, size, shape, surface features of the fruiting body, and host substrates, were recorded for taxonomic identification. Colours were assessed using the Methuen handbook of colour (Kornerup and Wanscher, 1967).

Fresh basidiomata (~50 mg) were washed with sterile deionised water within 1.5 mL microcentrifuge tubes by vortexing for 1 minute, followed by centrifugation at 13,000 rpm for 1 minute. The floating, cleaned basidiomata were separated from mangrove

sediment that had settled at the bottom. This washing step was repeated until no visible sediment remained. Genomic DNA was subsequently extracted from the cleaned basidiomata using the CTAB method described by O'Donnell et al. (1998). The internal transcribed spacer (ITS) and large subunit ribosomal RNA (LSU rDNA) regions were amplified using the primer pairs ITS1/ITS4 (White et al. 1990) and LR0R/LR7 (Vilgalys and Hester 1990), respectively. PCR reactions were performed in a total volume of 50 µL, containing 1× PCR BIO Taq Mix Red (PCR Biosystems, UK) and 0.5 µM of each primer. Amplification conditions followed the protocol described by Leetanasaksakul et al. (2024).

Raw DNA sequences were assembled using BioEdit v.7.0.5.3 (Hall 1999) and subsequently submitted to GenBank. Accession numbers for all sequences were provided in Table 1. Individual phylogenetic analyses were conducted for the ITS and LSU datasets, comprising 35 and 34 sequences, respectively. The dataset included cyphelloid species from nine genera (*Calathella*, *Cyphellopsis*, *Eoscyphella*, *Flagelloscypha*, *Henningsomyces*, *Lachnella*, *Merismodes*, *Nia*, and *Phaeosolenia*) and the non-cyphelloid species *Marasmiellus ramealis* TFB13755, which was used as the outgroup. Sequence data were obtained from previously published studies (Halama et al. 2019; Nakagiri et al. 2024) and downloaded from GenBank for phylogenetic tree construction. Sequence alignment was performed using MEGA X. Phylogenetic trees for both ITS and LSU datasets were constructed using the maximum likelihood method in MEGA X (Tamura et al., 2011), with 1,000 bootstrap replicates to assess branch support.

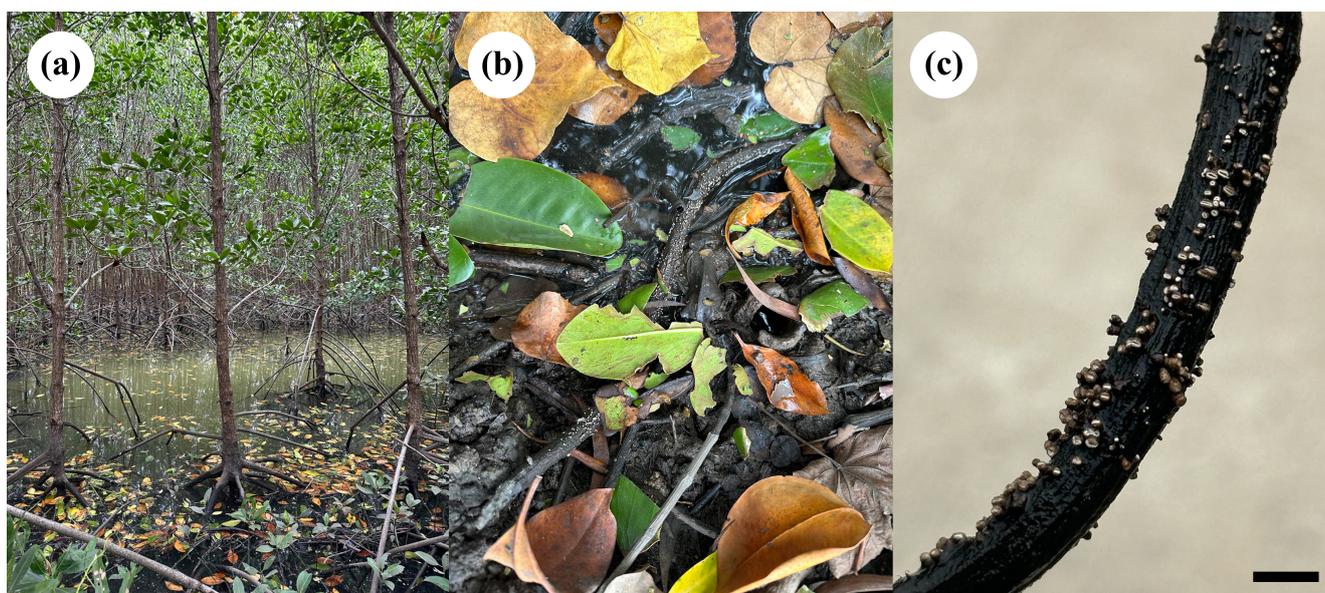
Specimens were dried at 45 °C for 48 to 72 hours and subsequently deposited in the BIOTEC Bangkok

Herbarium (BBH). The morphology of basidiocarps, basidia, and basidiospores was examined using a scanning electron microscope (SEM), following the methodology of Haranto et al. (2024). For SEM preparation, specimens were fixed overnight in 2% glutaraldehyde in 50 mM phosphate buffer (pH 7.2), then dehydrated through a graded ethanol series (10% to 100% at 10% increments) with 1-hour intervals. Samples were dried using a critical point drying technique and sputter-coated with gold before imaging with a Quanta 450 SEM (FEI Technologies Inc., Hillsboro, OR, USA).

For longitudinal sectioning, basidiocarps were also fixed in 2% glutaraldehyde in 50 mM phosphate buffer (pH 7.2) overnight. The tissues were dehydrated using a graded ethanol series, infiltrated with liquid paraffin (KemAus™, Elago Enterprises Pty Ltd, New South Wales, Australia), and embedded in surgipath paraplast (Leica Biosystems Richmond Inc., Illinois, USA). Sections were cut into 10 µm thick slices using a Leica RM2125 rotary microtome (Leica Biosystems Nussloch GmbH, Nussloch, Germany) and stained with 0.1% toluidine blue O in phosphate buffer pH 6.0 (Modified from Gupta and Pandey, 2013). Microscopic observations were performed using a light microscope (Nikon Instech Co., Ltd., Tokyo, Japan). Taxonomic data for the fungal specimens were also submitted to MycoBank.

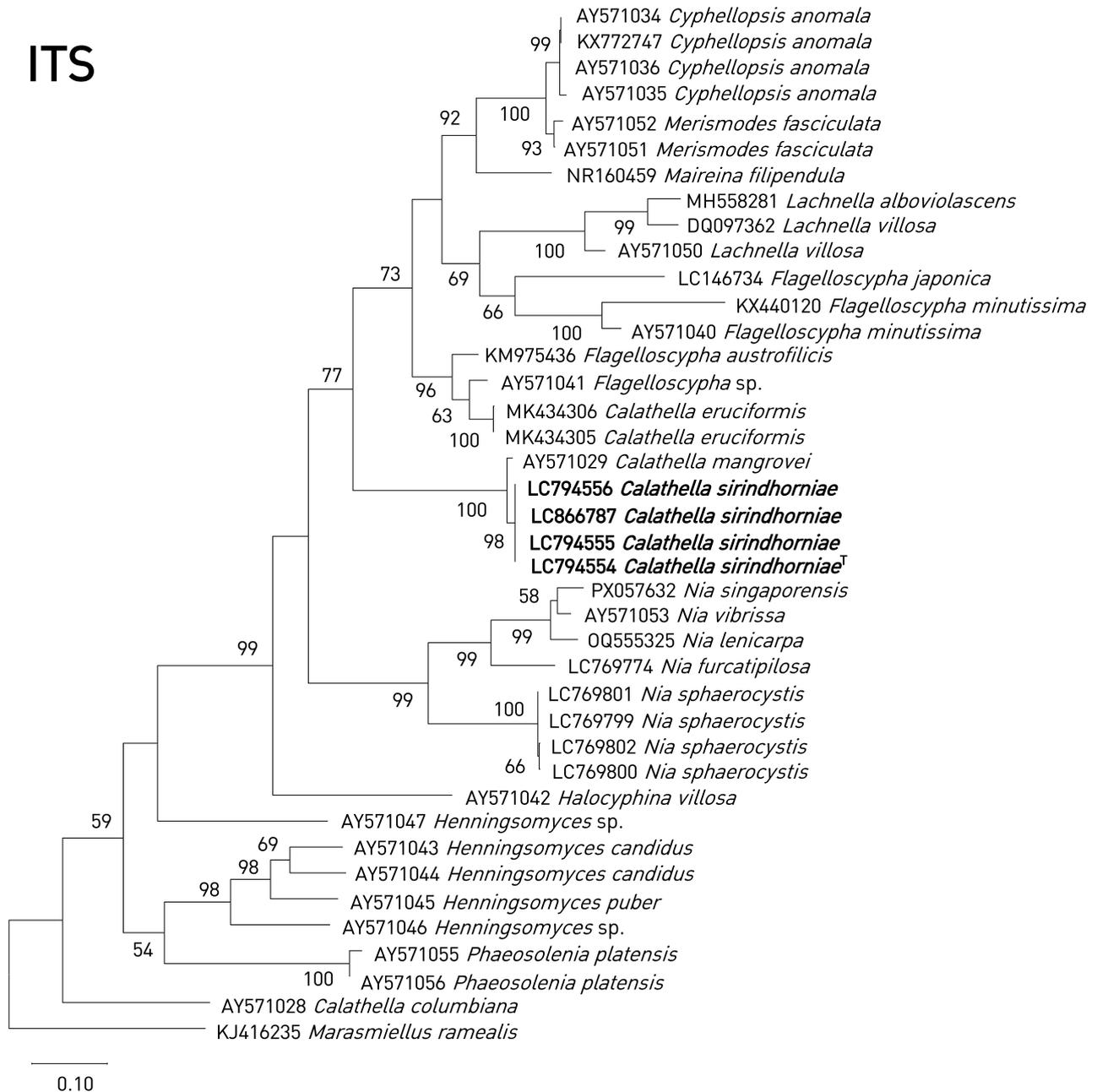
## RESULTS AND DISCUSSIONS

**Sample collection.**— The four specimens of novel *Calathella* species were found in a mangrove forest located in the intertidal zone. Specimens were discovered in two locations with similar ecological characteristics: the King Rama IX International Mangrove Botanical Garden, Mueang Chanthaburi District, Chanthaburi Province, and the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Ban Laem District, Phetchaburi Province, Thailand (Fig. 1 and Table 1). Both locations are dominated by *Rhizophora mucronata* (Rhizophoraceae) (Fig. 2A). The forest floor is rich in organic debris, particularly fallen leaves and hypocotyls of *R. mucronata*, which form a thick layer of detritus over the muddy substrate. Minute basidiomata were found exclusively on decaying hypocotyls of *R. mucronata* within the intertidal zone (Fig. 2B). These basidiomata were consistently observed on hypocotyls partially embedded in mangrove sediment and subjected to periodic submersion during tidal cycles. This pattern suggests that the fungus is saprobic, exhibiting a strong preference for a specific plant substrate and a notable tolerance to saline conditions. The basidiomata are minute and occur in dense clusters across the surface of the decaying hypocotyls of *R. mucronata* (Fig. 2C). The holotype samples were deposited in the BIOTEC Bangkok Herbarium (BBH) under accession number BBH 50085<sup>T</sup>.



**FIGURE 2.** Basidiomata and Ecology of *Calathella sirindhorniae*. **A.** The collection site for *Calathella sirindhorniae* is a mangrove reforestation area where *Rhizophora mucronata* is commonly planted as part of the King Rama IX International Mangrove Botanical Garden, Chanthaburi Province, Thailand. **B.** The basidiomata of *Calathella sirindhorniae* grow on the rotten hypocotyl of *Rhizophora mucronata* in the intertidal zone. **C.** A close-up view of the basidiomata on the rotten hypocotyl of *Rhizophora mucronata* is shown (Scale bar: 5 mm.).

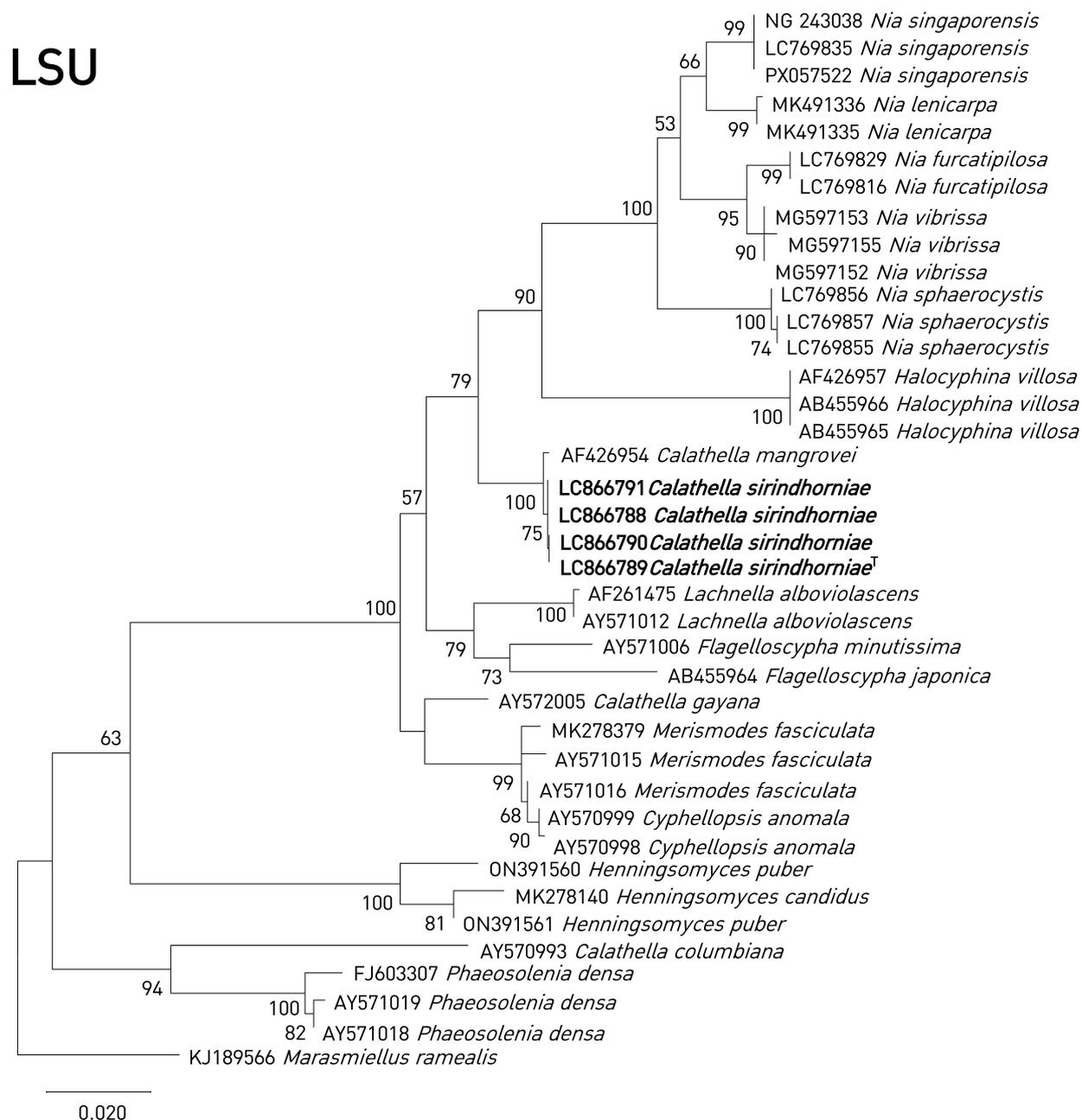
## ITS



**FIGURE 3.** The maximum likelihood (ML) phylogenetic tree of *Calathella sirindhorniae* was constructed using sequences of the ITS region, illustrating the position of the novel species in relation to closely related taxa. Bootstrap support values above 50% are displayed at the nodes. Strain numbers are listed after the species names, with strains from this study highlighted in bold. GenBank accession numbers for ITS sequences are provided. *Marasmiellus ramealis* TFB13755 was used as an outgroup. The scale bar represents a patristic distance of 0.10.

**Phylogenetic analyses.**— The ITS rDNA region sequences of the four specimens (DMKU-PKL2024-001 = BBH 50085<sup>T</sup>, holotype, DMKU-PKL2024-002, DMKU-PKL2024-003, and DMKU-PKL2025-001) yielded fragments of 769, 771, 771, and 684 bp, respectively. The LSU rDNA region sequences of the same specimens produced fragments of 1,315, 1,318, 1,326, and 1,318 bp, respectively. These sequences are available from GenBank under the accession numbers

listed in Table 1. Analysis of the LSU sequence indicates that the specimens are members of the genus *Calathella*. A BLASTn search against GenBank database revealed the closest match for the LSU sequence as *Nia lenicarpa* CBS H-23851 (GenBank accession no. MK491336), with 96% identity (1255 out of 1312 base pairs with 4 gaps). In addition, the ITS sequence showed the highest similarity to *C. mangrovei* strain 1-31-01 Jones (GenBank accession no. AY571029), with 96%

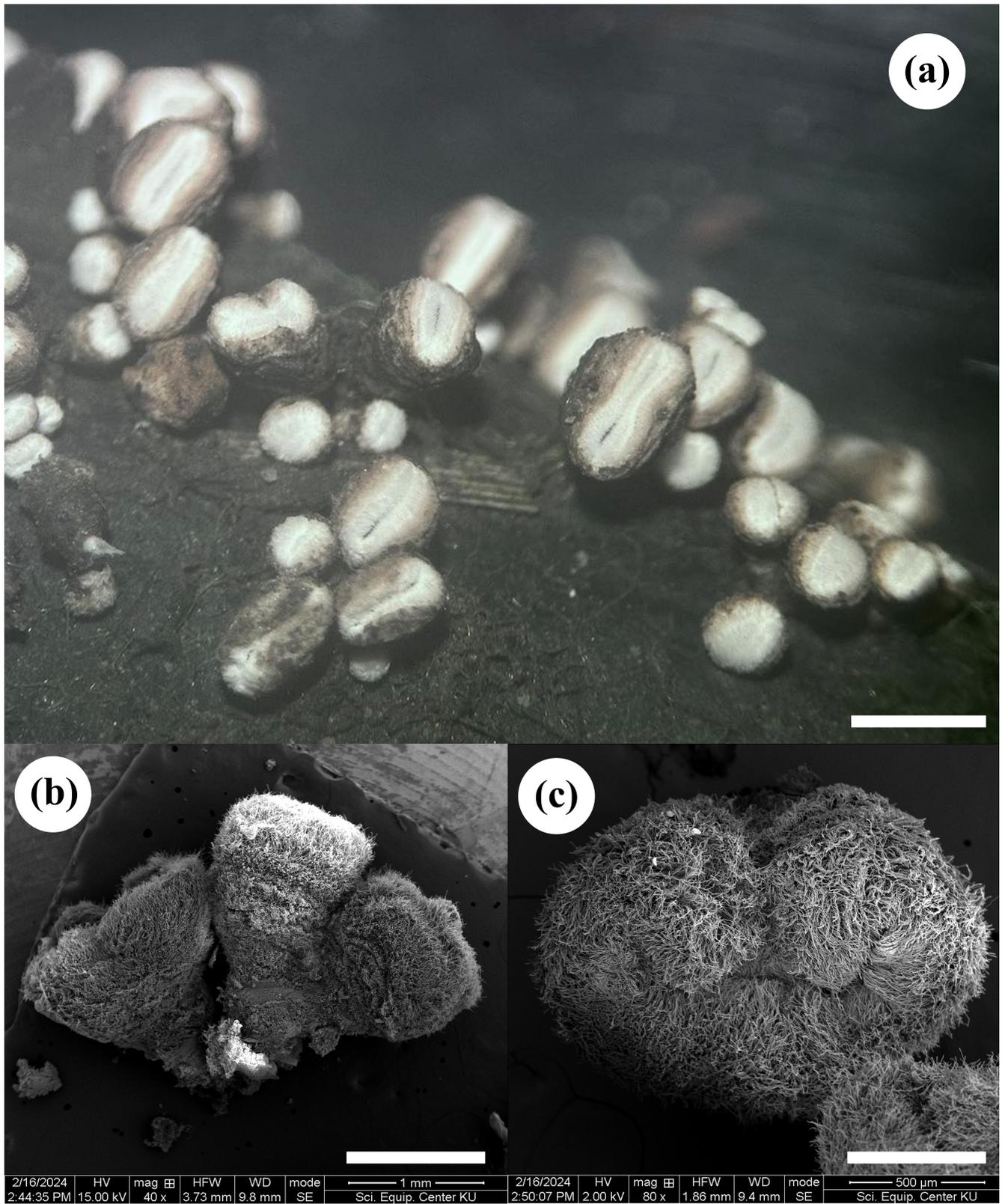


**FIGURE 4.** The maximum likelihood (ML) phylogenetic tree of *Calathella sirindhorniae* was constructed using sequences of the LSU region, illustrating the position of the novel species in relation to closely related taxa. Bootstrap support values above 50% are displayed at the nodes. Strain numbers are listed after the species names, with strains from this study highlighted in bold. GenBank accession numbers for LSU sequences are provided. *Marasmiellus ramealis* TFB13755 was used as an outgroup. The scale bar represents a patristic distance of 0.020.

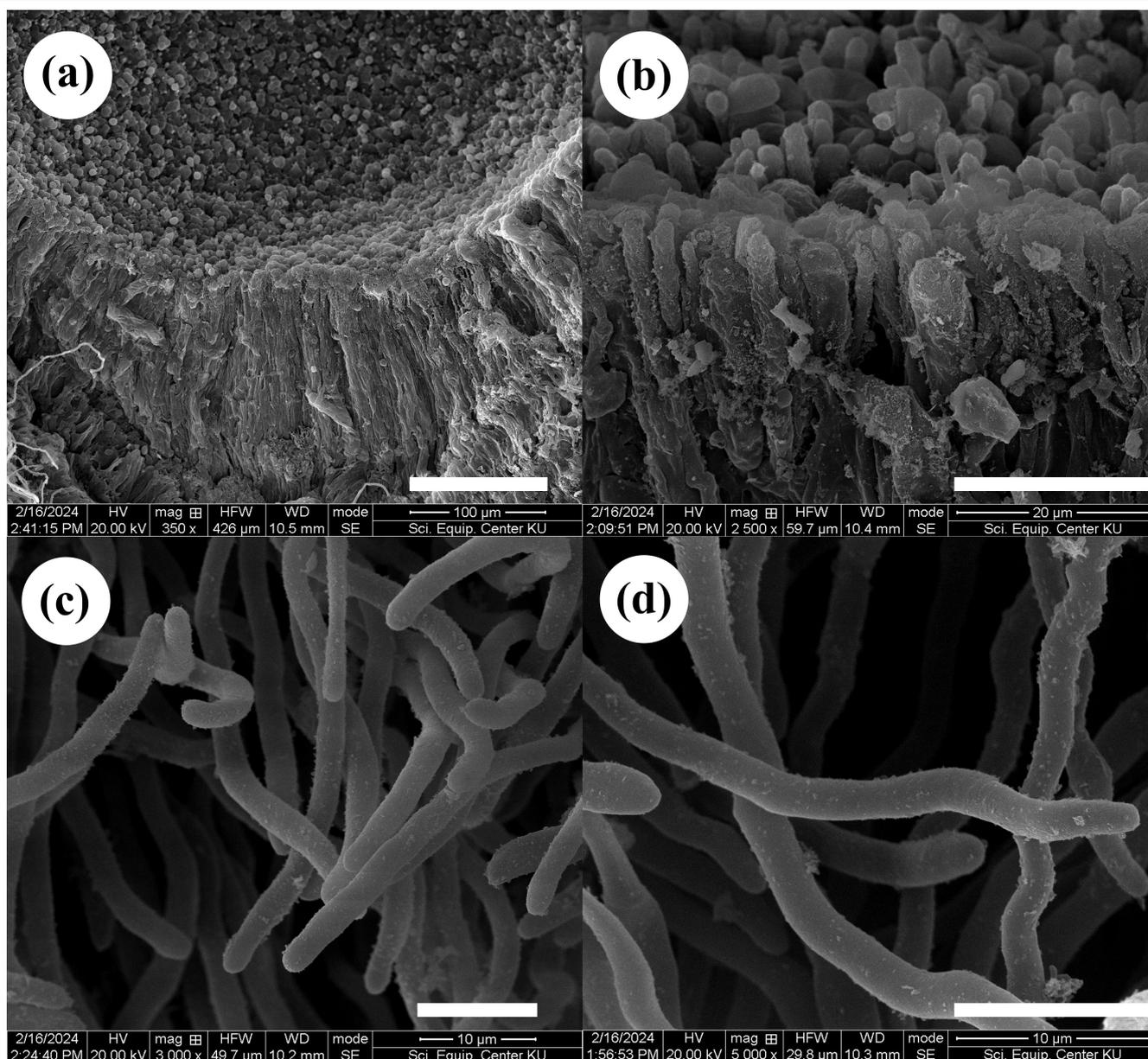
identity (662 out of 688 base pairs) 10 points nucleotide substitution and 15 gaps.

Phylogenetic analyses based on the ITS and LSU datasets (Figs 3, 4) consistently revealed that DMKU-PKL2024-001 = BBH 50085<sup>T</sup>, DMKU-PKL2024-002, DMKU-PKL2024-003, and DMKU-PKL2025-001 forms a distinct and well-supported lineage within the genus *Calathella*. In both trees, novel *Calathella* species clustered as a sister taxon to *C. mangrovei*,

with high bootstrap support (ITS = 100%, LSU = 100%), indicating a close but clearly separated evolutionary relationship. The congruent topologies of both gene regions strongly support the recognition of the novel *Calathella* species as an independent species. This genetic distinctness, together with its unique morphological characteristics, reinforces the delimitation of this novel *Calathella* as a novel species within *Calathella*.



**FIGURE 5.** *Calathella sirindhorniae* (BBH 50085, holotype). **A.** under a stereomicroscope, showing the overall structure. The basidiomata, composed of white hyphae, have a brown surface colouration primarily due to mangrove sediment. Scale bar: 1 mm. **B.** Side view of the basidiomata under the SEM, revealing detailed surface features and structural arrangement. Scale bar: 1 mm. **C.** Top view under the SEM, highlighting the surface morphology. Scale bar: 500  $\mu$ m.



**FIGURE 6.** *Calathella sirindhorniae* (BBH 50085, holotype). **A.** Longitudinal section of the basidiomata showing the hymenium and subhymenial layer. Scale bar: 100 μm. **B.** Detailed view of the hymenium. Scale bar: 20 μm. **C, D.** Marginal hairs. Scale bar: 10 μm.

### Taxonomy

#### *Calathella sirindhorniae* sp. nov.

Mycobank MB 858782

(Figs 5–8)

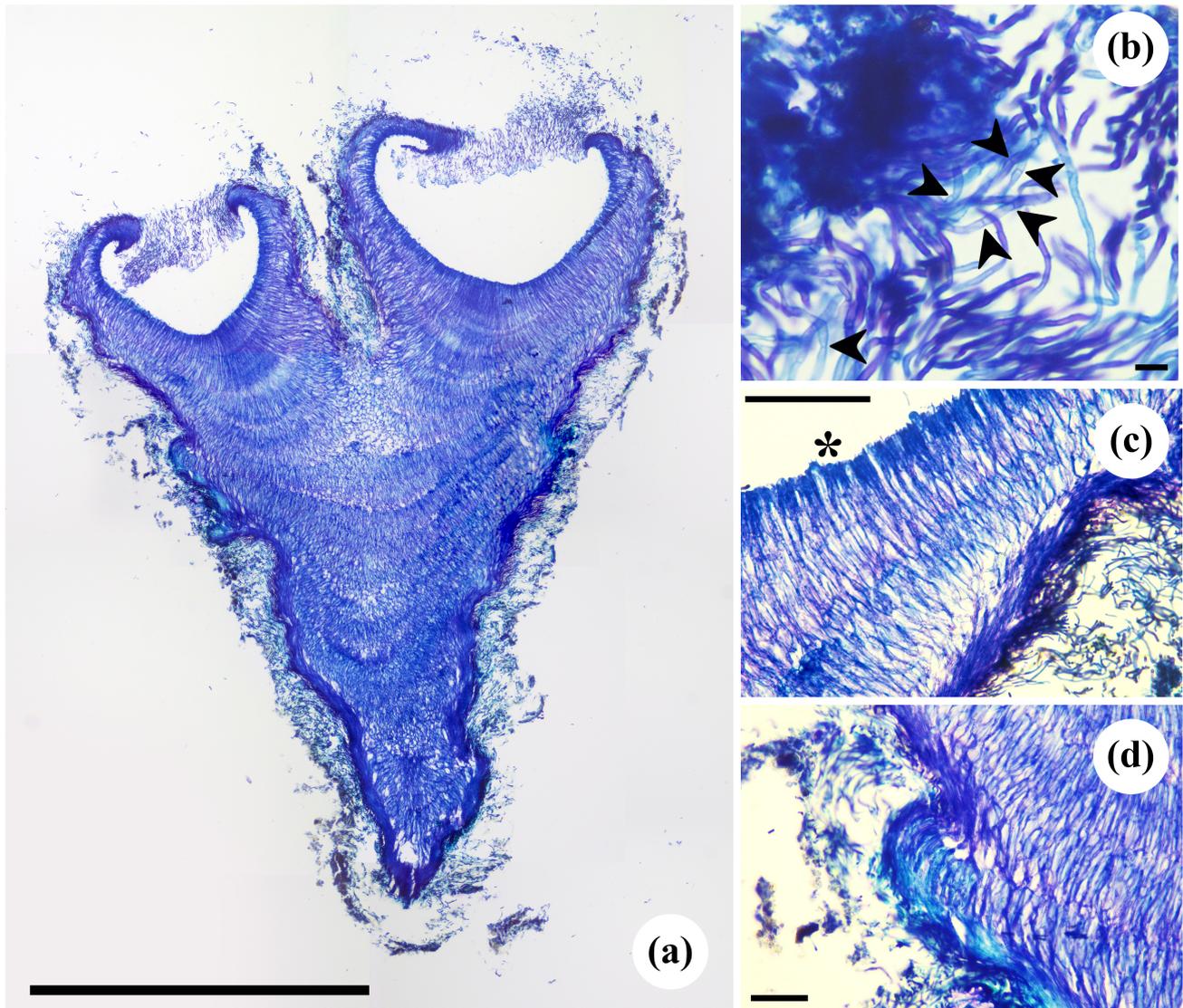
**Etymology.**— ‘*sirindhorniae*’ (si.rin.dhorn.ni.ae) this species is named to honour Her Royal Highness Princess Maha Chakri Sirindhorn, in recognition of her deep interest in natural history and her significant contributions to biodiversity and environmental conservation in Thailand.

**Common name.**— Sirindhorn cyphelloid fungi.

**Classification.**— *Calathella*, Marasmiaceae, Agaricales, Agaricomycetidae, Agaricomycetes, Agaricomycotina, Basidiomycota

**Holotype.**— Thailand: Chantaburi Province, Mueang Chanthaburi District, King Rama IX International Mangrove Botanical Garden on decaying hypocotyl of *Rhizophora mucronata*, 24 Dec. 2023, P. Lueangjaroenkit & C. Ngernsaengsaruy (DMKU-PKL2024-001 =BBH 50085, – holotype)

**Description.**— Basidiomata were gregarious, developing on the surface of decaying hypocotyls of *Rhizophora mucronata*, and appeared as scattered to clustered structures (Fig. 5A). The basidiomata were cup-shaped



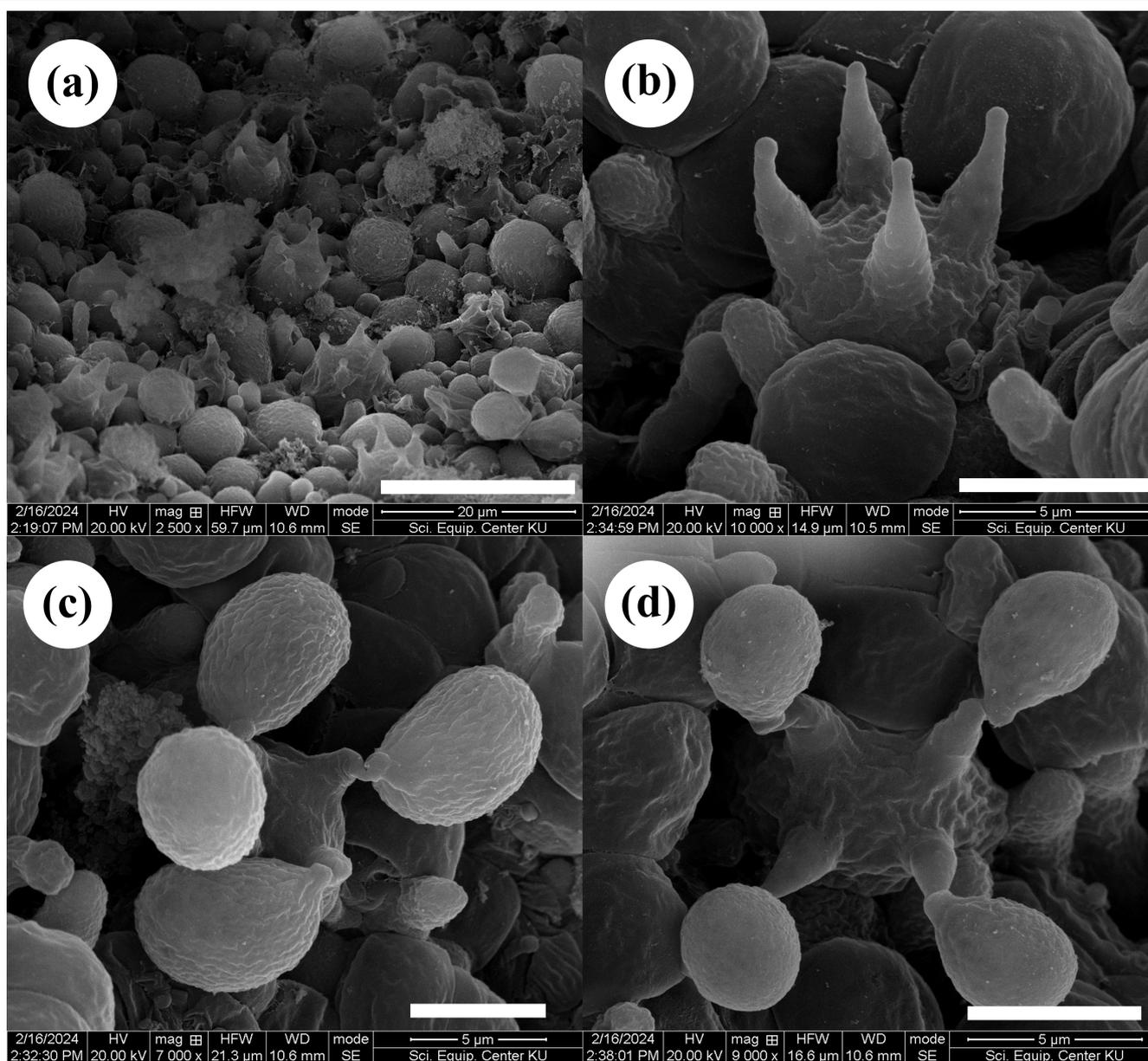
**FIGURE 7.** *Calathella sirindhorniae* (BBH 50085, holotype). **A.** Longitudinal section of the basidiomata. Scale bar: 1000  $\mu\text{m}$ . **B.** Marginal hyphae; arrowheads indicate septa without clamp connections. Scale bar: 10  $\mu\text{m}$ . **C.** Hymenium and subhymenium; asterisk indicate basidium with horn-like sterigmata. Scale bar: 100  $\mu\text{m}$ . **D.** Region showing old hymenium. Scale bar: 50  $\mu\text{m}$ .

to campanulate or turbate with a distinctly constricted base and a characteristic longitudinal slit. They exhibited hygroscopic behaviour, opening when wet and closing when dry. Dense colonies were often resulted from the proliferation of a single fruiting body, forming compact aggregations. (Fig. 5A). The basidiomata were initially white (1A1) to off-white due to a dense covering of white (1A1), hairy hyphae on the outer surface. However, in the natural mangrove habitat, they often appeared brownish as the external hyphae became stained by sediment and organic matter from the surrounding environment (Fig. 5A). The basidiomata measured (1.0–)1.3–1.7(–1.8) mm in height and (0.5–) 1.0–1.1(–1.3) mm in width, with the stalk region measuring 0.3–0.5 mm wide and the mid-region 0.7–0.8 mm wide. Dichotomous growth of basidiomata was

observed, resulting in branching or fused structures (Fig. 5B). No spore accumulation was detected on the apical hairs (Fig. 5C).

A cross-sectional view of the basidiomatal margin revealed a well-organised of hymenophore plane (Fig. 6A). The hymenial surface showed tightly packed basidia forming a compact hymenial layer without cystidia (Fig. 6B). Apical hairs (1.9–)2.0–2.3(–2.5)  $\mu\text{m}$ , somewhat curved with obtusely rounded tips. The surface was not smooth but covered with very fine encrustations. Clamp connections were not observed (Fig. 6C, D). In addition, the hairs not swelling in 5% KOH, dextrinoid.

A longitudinal section of the basidiomata of *Calathella sirindhorniae* revealed a shallow cupulate to turbate structure with a distinctly constricted base. The



**FIGURE 8.** *Calathella sirindhorniae* (BBH 50085, holotype). **A.** Surface view of the hymenial layer. Scale bar: 20 μm. **B.** Detailed view of a basidium bearing horn-like sterigmata. Scale bar: 5 μm. **C.** mature basidiospores with wrinkled surfaces. Scale bar: 5 μm. **D.** the attachment of the sterigma to the apiculus of young basidiospores on basidium. Scale bar: 5 μm.

apical hairs were slightly curved inward toward the hymenial surface. (Fig. 7A). The internal tissue (trama) displayed a distinctly layered arrangement, suggestive of gradual basidiomatal development through the sequential deposition of hyphal strata. The hyphae are thin- to moderately-walled, lack clamp connections, and were loosely to compactly arrange depending on the region and developmental stage. No clamp connection was observed on margin hairs (Fig. 7B). The subhymenium was composed of tightly interwoven hyphae forming a supportive layer beneath the hymenium. The hymenium consists of narrowly clavate basidia bearing horn-like sterigmata (Fig. 7C). An old hymenium layer is also visible (Fig. 7D), supporting the hypothesis of succes-

sive development of the basidioma through regeneration or continuation from a pre-existing basidiome.

The surface of the hymenium showed a dense layer of developing basidiospores. The surface is densely packed with various stages of basidiospore development, indicating active sporulation (Fig. 8A). Basidia were clavate with size (20–)23–26(–28) × (5.0–)6.1–8.3(–9.0) μm (n = 30), bearing conspicuous horn-like sterigmata; each basidium exhibits 4 sterigma (Fig 8B). The sterigmata appeared robust and sharply pointed. The sterigmata measured (3.3–)3.4–3.6(–3.8) μm in length, with a basal diameter of (1.4–)1.5–1.7(–1.8) μm and a distal (tip) diameter of 0.5–0.7 μm. Cystidia were absent. Basidiospores exhibited ellipsoid to ovoid

morphology with distinctly ornamented surfaces. The spore surface displayed longitudinal to irregularly reticulate ridges and grooves (Fig. 8C). Young basidiospores were attached to sterigmata, displaying a smoother surface texture compared to mature spores, indicating progressive surface ornamentation during spore maturation (Fig. 8D). The basidiospore size was (5.0–) 5.1–8.5(–8.9) × (3.2–)4.5–6.2(–6.7) μm, Q = 1.4–1.55, Qm = 1.47 ± 0.5, n = 30.

**Diagnosis.**— *Calathella sirindhorniae* can be distinguished from its closely related species, *C. mangrovei* Jones and Agerer (1992) by a combination of molecular, morphological, and ecological characteristics. The molecular analyses revealed ten nucleotide substitutions in the ITS and LSU rDNA regions, supporting its recognition as a distinct species. Morphologically, a distinguishing feature is the absence of clamp connections in *C. sirindhorniae*, which are consistently observed in *C. mangrovei*. The basidiospores of *C. sirindhorniae* are notably smaller and ornamented, and do not accumulate on the surface of the basidiomata. In contrast, *C. mangrovei* produces larger (8–12 × 6–10 μm), smooth-walled spores that typically accumulate on the tip of the basidiocarp. The basidiomata of *C. sirindhorniae* are generally shorter in height but wider in diameter compared to the distinctly tubular basidiomata of *C. mangrovei*, which range from 500–(800–1980)–2520 μm in height and 300–(400–990)–1080 μm in apical diameter. Ecologically, while both species inhabit mangrove environments, *C. sirindhorniae* has been found exclusively on the decaying hypocotyls of *Rhizophora mucronata*, suggesting a more substrate-specific ecology, whereas *C. mangrovei* occupies a broader range of mangrove-derived substrates. Moreover, *C. mangrovei* exhibits a distinct reddish-purple wood staining, which is absent in *C. sirindhorniae*.

**Distribution.**— The minute basidiomata were observed exclusively on decaying hypocotyls of *Rhizophora mucronata* within the intertidal zone of a mangrove forest predominantly composed of *R. mucronata*. The specimens have been found in both the eastern and western regions of the Gulf of Thailand.

#### ACKNOWLEDGEMENTS

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