

Research article

***Inocybe hopeae* sp. nov. and first record of *Pseudosperma keralense* (Inocybaceae) from Thailand**Bhavesh RAGHOONUNDON ¹, Santhiti VADTHANARAT ²,
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Abstract. Based on genetic studies, supported further by morphological and ecological differences, we present a taxonomic novelty (*Inocybe hopeae* Raghoonundon & Raspé sp. nov.) and a new geographical record (*Pseudosperma keralense*) from forests of Northern Thailand. *Inocybe hopeae* is characterized by medium-sized basidiomes, brownish orange to brown pileus that is darker towards the margin, off-white to pale brown context, light brown to dark brown stipe with off-white basal mycelium and pale brown to grayish brown lamellae. A three-gene phylogeny (LSU, *tef1*, *rpb2*) coupled with macroscopic/microscopic descriptions and illustrations are provided confirming the species' positions in their respective generic clades. *Inocybe hopeae* was sister to *I. thailandica* with strong support (BS = 100%, PP = 1.0). Our Thai collections of *ORI629* had similar morphological characters and 100% identical sequences with the holotype of *Pseudosperma keralense* from India.

Keywords. Diversity, molecular phylogeny, new species, taxonomy, Southeast Asia.

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Introduction

Inocybe (Fr.) Fr. (Inocybaceae Jülich, Agaricales Underw.) is a large genus with a total of 2249 taxon names listed in Index Fungorum (<http://www.indexfungorum.org/>). Recently, Matheny *et al.* 2019 raised the five major clades in *Inocybe* s. lat. to generic rank based on a six-locus phylogeny: *Inocybe* (Fr.) Fr. s. str., *Inosperma* (Kühner) Matheny & Esteve-Rav., *Mallocybe* (Kuyper) Matheny, Vizzini & Esteve-Rav., *Nothocybe* Matheny & K.P.D.Latha and *Pseudosperma* Matheny & Esteve-Rav. In addition to these five

genera, the Inocybaceae also include *Auritella* Matheny & Bougher and *Tubaratomyces* Esteve-Rav. & Matheny.

Inocybe s. str. remains the largest genus of Inocybaceae, comprising an estimated 850 species distributed worldwide (Matheny *et al.* 2019), with an increasing number of species being discovered as more areas are explored and morphological examinations of specimens are conducted (Deng *et al.* 2021; Li *et al.* 2021; Mešić *et al.* 2021). The presence of pleurocystidia and the basidiospores (typically with distinct apiculus) ranging from amygdaliform, elliptic, subcylindrical, angular, nodulose to spinose help distinguishing species of *Inocybe* from those of other genera in the family. *Inocybe* has a widespread distribution and occurs in all major biogeographic regions (Africa, Americas, Asia, Europe, Australasia) (Buyck & Eysartier 1999; Braaten *et al.* 2014; Horak *et al.* 2015; Pradeep *et al.* 2016, Bandini *et al.* 2019). They are commonly found in temperate areas (Singer 1962; Matheny *et al.* 2003) and less throughout the tropics (Horak 1980; Matheny *et al.* 2003).

The diversity of macrofungi in Thailand is very high but with limited documentation (Thongbai *et al.* 2018; Vadthananarat *et al.* 2021). During the past decade, several new species were described and taxa of Inocybaceae were newly recorded from Southeast Asia (Horak *et al.* 2015; Fan *et al.* 2018; Fan & Bau 2020). Thirty-nine species of *Inocybe* have been reported to occur in Thailand, thirteen of which were described as new species (Chandrasikul *et al.* 2011; Horak *et al.* 2015). In this study, we describe a new species of *Inocybe* s. str. and the first record of *Pseudosperma keralense* (K.P.D.Latha & Manim.) Matheny & Esteve-Rav. from Thailand.

Material and methods

Specimens collecting

Fresh basidiomes were collected during the rainy season (2019, 2020, 2021) from Chiang Mai and Chiang Rai Provinces, Northern Thailand. Collecting sites had an average temperature of 27.3°C, average rainfall of 122 mm and relative humidity of 74% (source: Thai meteorological department). The specimens were photographed on-site and wrapped in aluminium foil. Macroscopic descriptions were made on the same day, after which the specimens were dried in an electric dryer at 45–50°C. Specimens were deposited in the Mae Fah Luang University herbarium (MFLU).

Morphological, ecological, and taxonomic study

Each collection was described based on field notes and pictures of fresh basidiomes. Macro-chemical reactions on fresh basidiomes, habitat, locality information and elevation of collecting sites were recorded. Spore prints were taken for each collection. Colour codes follow Kornerup & Wanscher (1978). Microscopic structures were revived, examined and photographed in 10% potassium hydroxide (KOH) or 28–30% ammonium hydroxide (NH₄OH) solutions or 1% ammoniacal Congo red solution using a calibrated Nikon Y-TV55 camera fitted to a Nikon Eclipse Ni DIC microscope. A minimum of 60 basidiospores, 30 basidia, and 30 cystidia were randomly selected from the specimens and measured in KOH. The notation '[m/n/p]' represents the number of basidiospores 'm' measured from 'n' basidiomata of 'p' collections. Basidiospore measurements did not include the spines or nodules. The dimensions of the microscopic features are presented in the following format: '(a–)b–c–d(–e)', in which 'c' represents the average, 'b' the 5th percentile, 'd' the 95th percentile, and 'a' and 'e' the minimum and maximum values, respectively (Vadthananarat *et al.* 2018). Q, the length/width ratio, is presented in the same format. For scanning electron microscopy (SEM), a spore print was mounted onto an SEM stub with double-sided carbon tape. The sample was coated with gold, then examined and photographed with a JEOL JSM–5910 LV SEM (JEOL, Tokyo, Japan). A MycoBank number is provided for the new species.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from CTAB-preserved tissues or dry specimens using a CTAB isolation procedure adapted from Doyle & Doyle (1990). The LSU, ITS1–5.8S–ITS2 region of nuclear rDNA

(ITS) and parts of the genes *tef1* and *rpb2* were amplified by polymerase chain reaction (PCR). For PCR amplification, the primers used for nrLSU amplification were LR0R and LR5 (Vilgalys & Hester 1990). The ITS gene region was amplified using the primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). EF1-983F and EF1-2218R (Rehner & Buckley 2005) were used to amplify *tef1* and bRPB2-6F and bRPB2-7.1R (Matheny 2005) were used to amplify *rpb2*.

Sequence alignment and phylogenetic analysis

The sequences were assembled using Geneious 8 (Biomatters). In order to investigate the relationships of the new species with other species of *Inocybe*, the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) from GenBank was used to find the closest matches to the sequences. Reference sequences were downloaded and aligned using MAFFT ver. 7 (Kato & Standley 2013; <http://mafft.cbrc.jp/alignment/server/>). Introns were removed after the alignment based on the amino acid sequence of previously published sequences (Horak *et al.* 2015; Matheny *et al.* 2019). Before combining the data partitions, the topological incongruence between the datasets was assessed using maximum likelihood (ML) and Bayesian inference (BI) separately on each of the three-character sets, LSU, *tef1* exons, *rpb2* exons. The trees were examined for conflicts involving only nodes with ML bootstrap (BS) > 75% and Bayesian posterior probabilities (PPs) > 0.95 (Mason-Gamer & Kellogg 1996; Lutzoni *et al.* 2004). No significant incongruence was observed. Then, the concatenated three gene region matrix was prepared using BioEdit ver. 7.2.5 (Hall 2011).

Using jModeltest2 (Darriba *et al.* 2012) on XSEDE via the CIPRES Science Gateway, the best-fit model of substitution for analysis was estimated for each gene, based on the Bayesian Information Criterion (BIC). GTR + I + G was selected for both LSU and *rpb2* exons, while SYM + I + G was selected for *tef1* exons. Maximum likelihood (ML) phylogenetic tree inference was done with RAxML-HPC2 ver. 8.2.10 (Stamatakis 2006), using a mixed-model scheme and the GTR model of sequence evolution with 25 categories. Seven species of *Pseudosperma* and one species of *Nothocybe* were used as outgroup. Statistical support of the clades was obtained using nonparametric bootstrapping with 1000 replicates.

A Bayesian analysis was performed with the MrBayes ver. 3.2.7a algorithm (Ronquist *et al.* 2012), with LSU, *tef1* and *rpb2* data partitioned. The best-fit models for each character set were implemented as character set-specific models within partitioned mixed-model analyses of the combined dataset. Two simultaneous runs of five chains (four cold and one heated) were sampled every 200 generations until reaching the convergence parameters (standard deviation less than 0.01). The Bayesian analyses reached convergence after 1 500 000 generations. For all parameters, the average estimated sample size (ESS) value was over 200 and the average potential scale reduction factor (PRSF) value was greater than 1. The average standard deviation of split frequencies was 0.006923 at the end of the runs. The burn-in fraction (25%) was checked using Tracer ver. 1.7.1 (Rambaut *et al.* 2018). All model selection and phylogenetic analyses were done on the CIPRES Science Gateway (<https://www.phylo.org>; Miller *et al.* 2012).

The ITS1+ITS2 sequence of the specimen *ORI629* was blasted against GenBank database and compared to those of *Pseudospema keralense*, to confirm the conspecificity of our collections with the type of *P. keralense*. As already noticed by Matheny *et al.* (2019), the ITS region of Inocybaceae is characterized by high polymorphism and frequent indels, which makes the homology of characters difficult to ascertain. Therefore, this region was not included in the phylogenetic analyses.

Results

Phylogenetic analysis

The final multigene alignment comprised 57 OTUs and contained 2775 total nucleotide positions: 1140 positions from LSU (57 sequences), 945 positions from *tef1* (30 sequences), 690 positions from *rpb2* (56 sequences) (Table 1). Both the ML and Bayesian analyses produced the same tree topology; thus, only the ML tree is shown with both maximum likelihood bootstrap (MLB) and Bayesian posterior

Table 1 (continued on next page). List of collections used for DNA analyses, with origin, GenBank accession numbers and reference(s). The new species and new record are in bold.

Species	Specimen voucher	Origin	LSU	<i>tef1</i>	<i>rpb2</i>	References
<i>Inocybe angustifolia</i>	DED8139	Thailand	GQ892942	–	MH577422	Horak <i>et al.</i> 2015
<i>Inocybe angustifolia</i>	DED8146	Thailand	GQ892943	–	MH577421	Horak <i>et al.</i> 2015
<i>Inocybe affasterospora</i>	PBM2453	USA	AY702015	DQ435795	KM245975	Matheny <i>et al.</i> 2019
<i>Inocybe babruka</i>	CAL1344	India	KY549116	–	KY553237	Latha & Manimohan 2017
<i>Inocybe cacaocolor</i>	PBM3790	Australia	KJ756464	–	KJ756422	Matheny <i>et al.</i> 2019
<i>Inocybe corydalina</i>	TURA6488	Belgium	AY038314	–	AY337370	Matheny <i>et al.</i> 2019
<i>Inocybe flavoalbida</i>	PBM3768	Australia	KJ729901	MK426183	KJ729932	Matheny <i>et al.</i> 2019
<i>Inocybe flavosquamulosa</i>	CAL1353	India	KY549117	–	KY553238	Latha & Manimohan 2017
<i>Inocybe floccosistipitata</i>	CAL1256	India	KY549119	–	KY553239	Latha & Manimohan 2017
<i>Inocybe fuscicothurnata</i>	PBM3980	USA	KY990485	MK426184	MF416408	Matheny <i>et al.</i> 2019
<i>Inocybe hopeae</i> sp. nov.	ORI632	Thailand	ON831502	ON553694	ON553691	this study
<i>Inocybe hopeae</i> sp. nov.	ORI665	Thailand	ON831503	ON553695	ON553692	this study
(holotype)						
<i>Inocybe humidicola</i>	PBM3719	Australia	KJ801181	MK426185	KJ811575	Matheny <i>et al.</i> 2019
<i>Inocybe hydrocybiformis</i>	TBGT12318	India	KP170911	–	KM245987	Pradeep <i>et al.</i> 2016
<i>Inocybe hydrocybiformis</i>	CAL1376	India	KY549121	–	KY553240	Latha & Manimohan 2017
<i>Inocybe insulana</i>	CAL1258	India	KY549122	–	KY553241	Latha & Manimohan 2017
<i>Inocybe iringolkavensis</i>	K(M)191731	India	KM924519	–	KY553242	Latha & Manimohan 2017
<i>Inocybe kurkuriya</i>	CAL1352	India	KY549125	–	KY553245	Latha & Manimohan 2017
<i>Inocybe kuruvensis</i>	K(M)191734	India	KM924517	–	KY553246	Latha & Manimohan 2017
<i>Inocybe lanuginosa</i>	PBM956	USA	KP170923	MK426186	KM245992	Matheny <i>et al.</i> 2019
<i>Inocybe lasseroides</i>	PBM3749	Australia	KP170924	MK426187	KM245993	Matheny <i>et al.</i> 2019
<i>Inocybe lineata</i>	DED8048	Thailand	GQ892958	–	KM245999	Horak <i>et al.</i> 2015
<i>Inocybe luteifolia</i>	PBM2642	USA	EU307814	MK426188	EU307816	Matheny <i>et al.</i> 2019
<i>Inocybe magnifolia</i>	MCA2441	Guyana	JN642244	MK426189	EU600899	Matheny <i>et al.</i> 2019
<i>Inocybe melanopus</i>	PBM3975	USA	MH220276	MK426190	MH249807	Matheny <i>et al.</i> 2019
<i>Inocybe muthangensis</i>	K(M)191735	India	KM924516	–	KY553247	Latha & Manimohan 2017
(holotype)						
<i>Inocybe pallidicremea</i>	PBM2039	USA	AY380385	MK426191	AY337388	Matheny <i>et al.</i> 2019
<i>Inocybe papilliformis</i>	TBGT10480	India	KP170912	–	KM245988	Pradeep <i>et al.</i> 2016
<i>Inocybe parvibulbosa</i>	DED8021	Thailand	GQ892954	–	KM555134	Horak <i>et al.</i> 2015
<i>Inocybe persicinipes</i>	PBM2197	Australia	EU600837	MK426192	EU600836	Matheny <i>et al.</i> 2019
<i>Inocybe pileosulcata</i>	TBGT10742	India	KP170979	MK426193	KM406218	Matheny <i>et al.</i> 2019
<i>Inocybe pingala</i>	CAL1345	India	KY549130	–	KY553248	Latha & Manimohan 2017
(holotype)						
<i>Inocybe pluvialis</i>	PBM3228	Australia	KF853401	MK426194	KF891954	Matheny <i>et al.</i> 2019
<i>Inocybe purpureoflavida</i>	TBGT3388	India	KT329457	–	KT329445	Pradeep <i>et al.</i> 2016
<i>Inocybe relicina</i>	JV10258	Finland	AY038324	–	AY333778	Matheny <i>et al.</i> 2019
<i>Inocybe roseifolia</i>	CO5576	USA	MK421968	MK426195	MH577441	Matheny <i>et al.</i> 2019
<i>Inocybe rufobadia</i>	NLB885	Australia	KF915290	MK426196	KF991385	Matheny <i>et al.</i> 2019
<i>Inocybe serrata</i>	PBM3235	Australia	KP171012	MK426197	KM555111	Matheny <i>et al.</i> 2019
<i>Inocybe snigdha</i>	CAL1350	India	KY549135	–	KY553250	Latha & Manimohan 2017
(holotype)						
<i>Inocybe spadicea</i>	PBM2203	Australia	EU600865	MK426198	–	Matheny <i>et al.</i> 2019
<i>Inocybe stellata</i>	DED8015	Thailand	GQ892966	–	KM656106	Horak <i>et al.</i> 2015
<i>Inocybe stellata</i>	DED8162	Thailand	GQ892961	–	KM656104	Horak <i>et al.</i> 2015
<i>Inocybe subexilis</i>	PBM2620	USA	EU307845	MK426199	EU307847	Matheny <i>et al.</i> 2019
<i>Inocybe thailandica</i>	DED8049	Thailand	GQ892968	MK426200	KM656129	Matheny <i>et al.</i> 2019
<i>Inocybe torresiae</i>	PBM2157	Australia	EU600874	–	EU600873	Matheny <i>et al.</i> 2019
<i>Inocybe tubarioides</i>	PBM2550	USA	AY732211	MK426201	EU307855	Matheny <i>et al.</i> 2019

Table 1 (continued). List of collections used for DNA analyses, with origin, GenBank accession numbers and reference(s). The new species and new record are in bold.

Species	Specimen voucher	Origin	LSU	<i>tef1</i>	<i>rpb2</i>	References
<i>Inocybe viraktha</i>	CAL1357	India	KY549137	–	KY553252	Latha & Manimohan, 2017
<i>Inocybe wayanadensis</i>	K(M)191737	India	KM924515	–	KY553254	Latha & Manimohan, 2017
<i>Nothocybe distincta</i>	ZT9250	India	EU604546	MK426212	EU600904	Matheny <i>et al.</i> 2019
<i>Pseudosperma bulbosissimum</i>	DBG19916	USA	MH024885	MK426213	MH249788	Matheny <i>et al.</i> 2019
<i>Pseudosperma cercocarpi</i>	BK2006986	USA	EU600890	MK426214	EU600889	Matheny <i>et al.</i> 2019
<i>Pseudosperma keralense</i>	ORI629	Thailand	ON831501	ON553693	ON553690	this study
<i>Pseudosperma keralense</i>	TBGT12854	India	KP171059	–	KM656098	Pradeep <i>et al.</i> 2016
<i>Pseudosperma mimicum</i>	PBM2574	USA	EF561633	MK426215	EU307858	Matheny <i>et al.</i> 2019
<i>Pseudosperma notodryinum</i>	CO4463	USA	MK421970	MK426216	MH577509	Matheny <i>et al.</i> 2019
<i>Pseudosperma sororium</i>	PBM3901	USA	MH220278	MK426218	MH249810	Matheny <i>et al.</i> 2019
<i>Pseudosperma spurium</i>	BK180809723	USA	EU600868	MK426219	EU600867	Matheny <i>et al.</i> 2019

probabilities (BPP) values (Fig. 1). In the obtained tree, the new species *Inocybe hopeae* Raghoonundon & Raspé sp. nov. shared a sister relationship with the holotype of *Inocybe thailandica* E.Horak, Matheny & Desjardin (voucher *DED8049*), providing strong statistical support (MLB/BPP = 100/1.00). Both the paratype (*ORI632*) and holotype (*ORI665*) of *Inocybe hopeae* had 100% identical LSU, *tef1* and *rpb2* sequences.

The specimen *ORI629* yielded a fungal ITS sequence 100% identical to the holotype of *Pseudosperma keralense* (voucher *K(M)191712*, sequence KM924523) from India, revealing the presence of this species in Thailand. The *rpb2* sequence of *ORI629* was also found to be similar to that of *P. keralense* holotype (sequence KY553243), with 4 heteromorphisms in the sequences of *ORI629* (Table 2).

Taxonomic treatments

Phylum Basidiomycota R.T.Moore
 Class Agaricomycetes Doweld
 Order Agaricales Underw.
 Family Inocybaceae Jülich
 Genus *Inocybe* (Fr.) Fr.

Inocybe hopeae Raghoonundon & Raspé sp. nov.
 MycoBank: MB844101
 Figs 2–4

Diagnosis

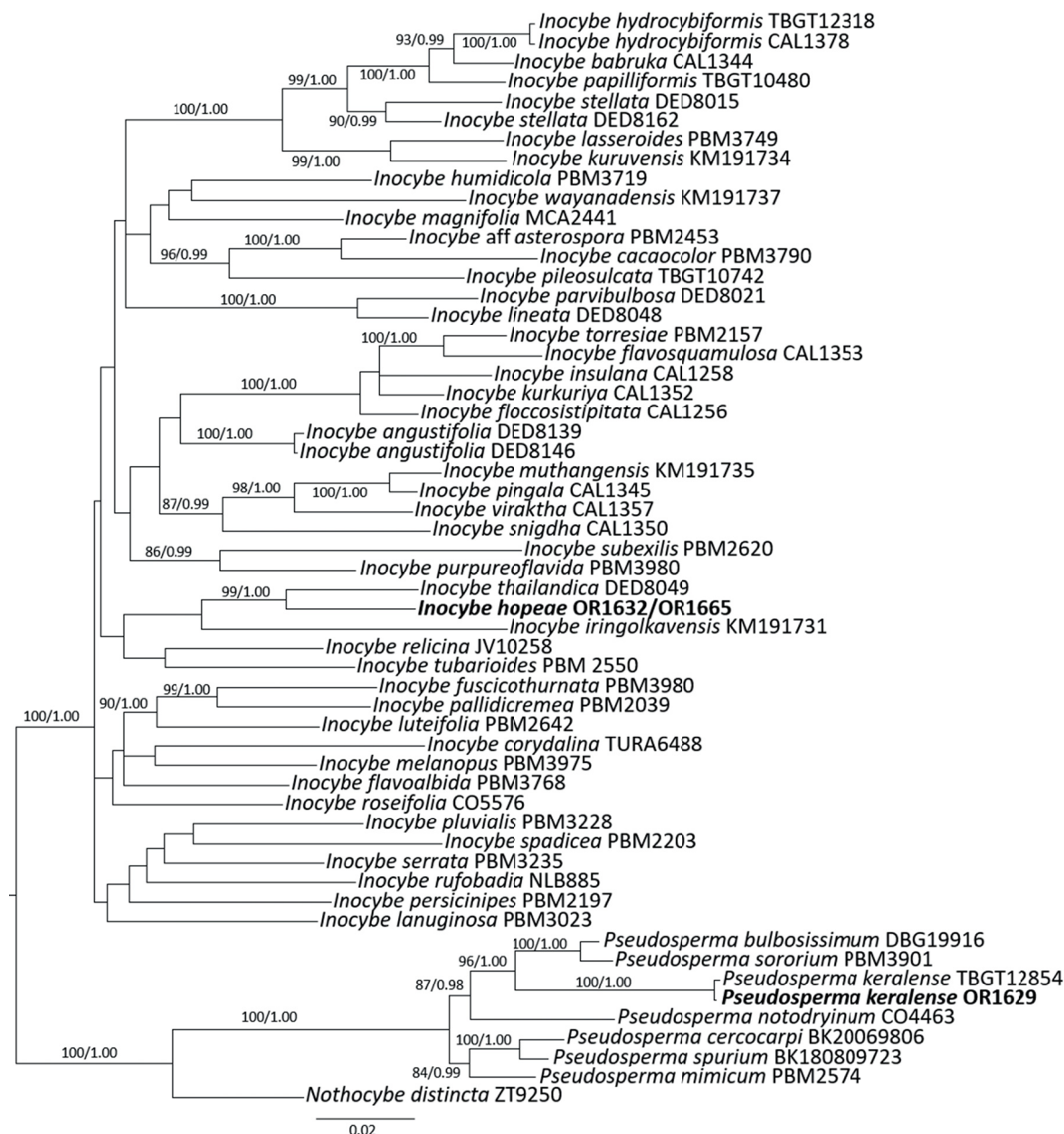
Inocybe hopeae sp. nov. is distinguished from *Inocybe thailandica* by having wider brownish orange to brown pileus darker towards the margin, thicker off-white to pale brown context, light brown to dark brown stipe, pleurocystidia being different from cheilocystidia, broader and longer basidia and caulocystidia.

Etymology

The epithet ‘*hopeae*’ refers to being found under the tree *Hopea odorata* Roxb.

Table 2. Heteromorphism positions in *OR1629* when compared with the holotype of *Pseudosperma keralense* (K.P.D.Latha & Manim.) Matheny & Esteve-Rav. (*K(M)191712*).

Positions	66	102	124	267
<i>OR1629</i>	Y	R	Y	Y
<i>K(M)191712</i>	C	A	T	C

**Fig. 1.** Maximum likelihood phylogenetic tree inferred from the three gene dataset (*tef1*, LSU, *rpb2*). Seven species of *Pseudosperma* Matheny & Esteve-Rav. and one species of *Nothocybe* Matheny & K.P.D.Latha were used as outgroup taxa. Maximum likelihood bootstrap (MLB, left) $\geq 70\%$ and Bayesian posterior probabilities (BPP, right) ≥ 0.95 are shown above supported branches. The new species and new record are in bold.

Material examined

Holotype

THAILAND • Chiang Rai Province, Mueang district, Mae Fah Luang University; 20°02'54" N, 99°53'30" E; 427 m a.s.l.; 30 Jun. 2020; *O. Raspé*, OR 1665; GenBank accessions: LSU ON831503, *tef1* ON553695, *rpb2* ON553692; MFLU[MFLU22-0084].

Additional material

THAILAND • Chiang Rai province, Mueang district, Mae Fah Luang University; 20°02'47" N, 99°53'41" E; 442 m a.s.l., 20 Apr. 2021; *B. Raghoonundon*, BR244; MFLU[MFLU22-0082] • Chiang Rai Province, Pa daet; 19°34'15" N, 100°00'23" E; 603 m a.s.l., 6 Sep.2019; *O. Raspé*, OR1630; MFLU[MFLU22-0085] • same collection data as for preceding; *O. Raspé* OR1632; MFLU[MFLU23-0076].

Description

Pileus 11–51 mm in diameter, at first obtusely conical, becoming umbonate to plano-umbonate when mature, margin deflexed; surface dull and dry, fibrillose to striate with recurved scales, brownish orange (5C4–5C7) to brown (6D5–6E6), dark brown (6F6–7F6) near the margin; thin context 2–4 mm, off-white to pale brown (6A1–6C4), unchanging when bruised, solid and fleshy. Stipe central, cylindrical, 33–94 mm × 2–7 mm, slightly bulbous; surface even, dull and dry, with minute squamules at the top, elsewhere glabrous, light brown (6D4–7D5) to dark brown (7F5), darker towards the top, unchanging when bruised; basal mycelium off-white (6A1), turning orange when bruised (6A7–6A8); context solid or fibrillose, fleshy, pale brown (6C4), staining slightly orange (5A3–5A4) when bruised. Hymenophore lamellate, adnexed to adnate with a decurrent tooth; lamellae 35–54 reaching stipe, 5–7 lamellulae, close, thick, ventricose, forked mostly near the margin, yellowish gray (4B1–4B2), becoming brown (5D5–6E5) with age, lamellae edge even. Smell and taste not distinctive.

Basidiospores [125/6/4] (5.3–)5.8–8.2–11.1(–14) µm in diam., globose to ovoid, with conical-spinose projections up to 3.9 µm long, sub-globose to globose, brownish in KOH and NH₄OH. Basidia (33.2–)33.9–44–51.9(–52.3) × (9.8–)10.1–14.6–17.6(–17.7) µm, 4-spored, clavate, hyaline, sterigmata up to 8 µm long. Cheilocystidia (39–)39.5–47.3–71(–71) × (10.2–)10.2–14.9–20.2(–20.2) µm, frequent, thick-walled, fusiform to broadly fusiform with some scattered crystals at the top, hyaline, yellowish in KOH and NH₄OH. Pleurocystidia (27.6–)29.1–41.9–50.1(–54.7) × (9.2–)9.4–12.9–15.2(–16.5) µm, fusiform, thick-walled, hyaline, yellowish in KOH and NH₄OH. Pileipellis an intricate trichoderm of interwoven cylindrical hyphae 4–6.2–8 µm wide, terminal cells conical to fusiform, wall encrusted with yellowish brown pigments; subpellis hyphae 11–14.5–22 µm wide, broadly cylindrical, encrusted with yellowish brown pigments. Caulocystidia (32–)34–44.9–57.8(–58.1) × (13.7–)14.5–18.5–21.4(–23.2) µm, polymorphic, clavate to fusiform, thick-walled, hyaline, yellowish in KOH and NH₄OH, rarely with crystals. Stipitipellis a trichoderm of interwoven hyphae 3–4.9–8 µm wide, partially composed of clavate cells, slightly brownish in KOH. Clamp connections present.

Habitat

Scattered on soil under *Hopea odorata*; in tropical forest dominated by Dipterocarpaceae Blume (*Dipterocarpus* spp. and *Shorea* spp.), with some Fagaceae Dumort. (*Quercus* spp., *Lithocarpus* spp. and *Castanopsis calathiformis* Rehder & E.H.Wilson). Also found under planted *Hopea odorata*.

Known distribution

Thus far known only from northern Thailand.

Notes

Inocybe hopeae sp. nov. is macro-morphologically characterized by medium sized basidiomes, brownish orange to brown pileus becoming darker towards the margin, off-white to pale brown context, light brown to dark brown stipe with off-white basal mycelium and pale brown to grayish brown lamellae. Molecular



Fig. 2. a. Basidiomes of *Inocybe hopeae* Raghoonundon & Raspé sp. nov. (OR1665, holotype). c–d. Basidiomes of *Inocybe hopeae* (OR1630). b, e–f. Basidiomes of *Pseudosperma keralense* (K.P.D.Latha & Manim.) Matheny & Esteve-Rav. (OR1629). Scale bars: a = 10 mm, b–d = 5 mm.

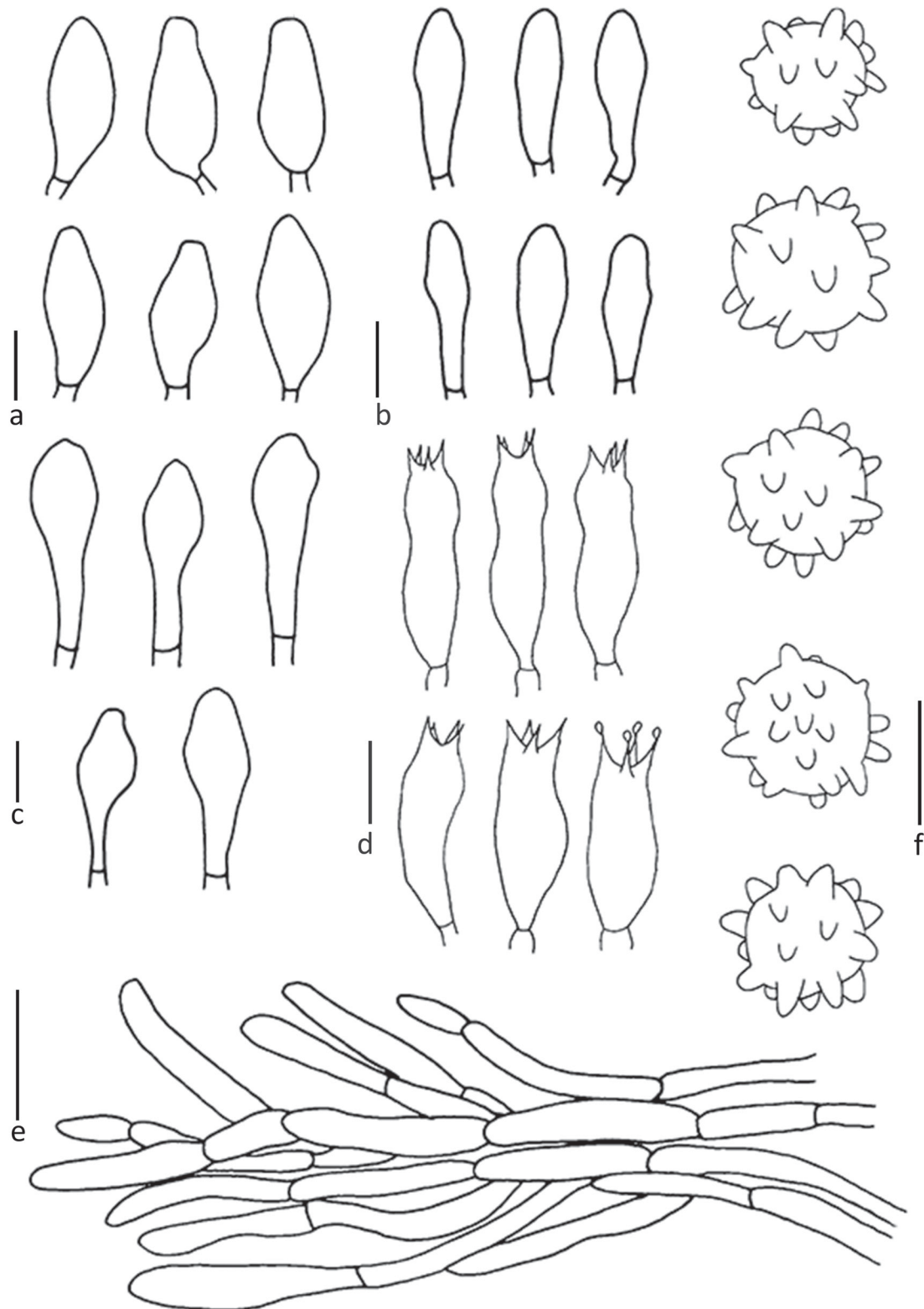


Fig. 3. Microscopic features of *Inocybe hopeae* Raghoonundon & Raspé sp. nov. **a.** Caulocystidia. **b.** Pleurocystidia. **c.** Cheilocystidia. **d.** Basidia. **e.** Pileipellis. **f.** Basidiospores. Scale bars: a–e = 20 μ m, f = 10 μ m.

data (Fig. 1) confirmed a close relationship between *I. hopeae* and another species from Thailand, *I. thailandica*. However, when compared to the latter, our species is morphologically different. *Inocybe thailandica* has a dark brown pileus becoming pale brown towards the margin, which is the opposite of *I. hopeae*, in which the brownish orange pileus is darker towards the margin. Moreover, *I. hopeae* has a wider pileus and thicker context as compared to *I. thailandica*. Finally, *I. thailandica* has a light brown stipe that is darker towards the base, whereas in *I. hopeae*, the stipe is darker brown towards the apex.

The micro-morphological characters of *I. hopeae* sp. nov. somewhat resemble those of *I. thailandica*. However, upon closer examination, *I. hopeae* have broader and longer basidia ($33.2\text{--}52.3 \times 9.8\text{--}17.7 \mu\text{m}$) compared to *I. thailandica* ($28\text{--}36 \times 7\text{--}9 \mu\text{m}$). The caulocystidia of *I. hopeae* ($32\text{--}58.1 \times 13.7\text{--}23.2 \mu\text{m}$) also are longer and broader than those of *I. thailandica* ($20\text{--}36 \times 6\text{--}12 \mu\text{m}$). Furthermore, the cheilocystidia of *I. thailandica* ($30\text{--}40 \times 10\text{--}20 \mu\text{m}$) are shorter than those in the new species ($39\text{--}71 \times 10.2\text{--}20.2 \mu\text{m}$). Additionally, Horak *et al.* (2015) mentioned the pleurocystidia and cheilocystidia of *I. thailandica* are similar, which is not the case in *I. hopeae*, whereby the pleurocystidia are narrower and shorter than the cheilocystidia. The basidiomes of *I. thailandica* are also putative associates of *Castanopsis* (D. Don) Spach (Horak *et al.* 2015) as compared to *I. hopeae*, which was found in forests dominated by Dipterocarpaceae with some Fagaceae or growing in association with planted *Hopea odorata*.

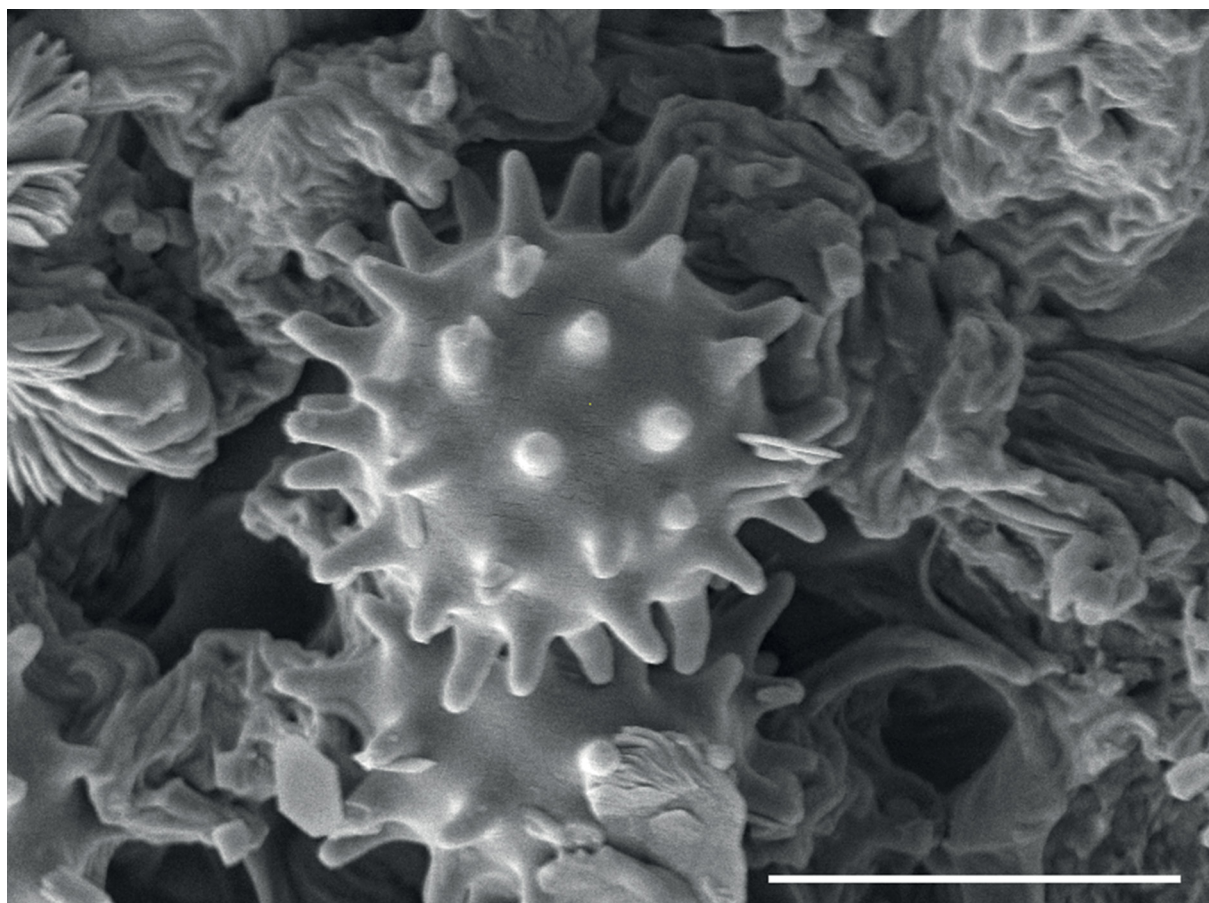


Fig. 4. Scanning electron micrograph of basidiospores from the holotype of *Inocybe hopeae* Ragoonundon & Raspé sp. nov. (ORI665). Scale bar = 10 μm .

Genus *Pseudosperma* Matheny & Esteve-Rav.

Pseudosperma keralense (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.

Mycobank: MB830515

Index Fungorum: IF830515

Figs 2, 5

≡ *Inocybe keralensis* K.P.D.Latha & Manim., *Mycologia* 108 (1): 116 (Latha & Manimohan 2016) (basionym).

≡ *Inocybe rimulosa* C.K.Pradeep & Matheny, *Mycological Progress* 15 (3/24): 12 (Pradeep *et al.* 2016).

Material examined

THAILAND • Chiang Rai Province, Pa Daet; 19°34'15" N, 100°00'23" E; 603 m a.s.l.; 6 Sep. 2019; *O.Raspé* OR1629; GenBank accessions: ITS ON383911, LSU ON831501, *tef1* ON553693, *rpb2* ON553690; MFLU[MFLU22-0083].

Description

Pileus 11–15 mm in diameter, convex to plano-convex, slightly depressed around the centre with a small obtuse umbo, margin almost straight, slightly uplifted at some places; surface dull and dry, radially rimose, becoming fibrillose near the margin, brownish orange (5C6–5C8) at the centre to light brown (6C4–6C5) the margin; thin context 1–2 mm, off-white (6A1), unchanging when bruised; soft and fleshy. Stipe central, cylindrical, 30–35 × 1–3 mm, slightly tapering toward the apex; surface dull and dry, fibrillose throughout, finely pruinose towards the apex, cream, brownish orange (6C3) towards the base; slightly enlarged and whitish base, basal mycelium off-white; context hollow, thin, white. Hymenophore lamellate, adnexed to adnate with a decurrent tooth; lamellae close, thick, slightly wavy, grayish orange (5B3) to light brown (5D4–6D4), becoming brown (6E5–6E8) with age, lamellae edge serrate. Smell and taste not distinctive.

Basidiospores [120/1/1] (8.6–)8.8–11–13.2(–13.4) × (6.7–)6.9–7.8–8.6(–8.8) μm, Q = (1.28–)1.28–1.4–1.53(–1.53), smooth, ellipsoid to phaseoliform, slightly thick-walled, yellowish brown. Basidia (22.2–)22.9–28.3–32.6(–33.5) × (11.2–)11.3–12.9–14.4(–14.6) μm, clavate, thin-walled, pale yellowish brown, 4-spored, sterigmata up to 5 μm long. Cheilocystidia (19.3–)19.6–27.5–31.8(–35.7) × (8.7–)9.2–11.7–13.7(–14.6) μm, abundant, clavate to fusiform, thin to thick-walled, hyaline, pale yellow in KOH or NH₄OH, rarely with encrustations. Pleurocystidia absent. Pileipellis a double layered cutis with a first one superficially disrupted, composed of thin- to slightly thick-walled, smooth hyphae 5–8.9–13 μm wide with a yellowish-brown spiral encrustation. Caulocystidia absent. Stipitipellis a cutis with disrupted loose hyphae with cystoid terminal cell at the stipe apex; hyphae 5–7.1–10 μm wide, thin to thick-walled, pale yellow or brownish yellow in KOH, encrusted with yellowish brown contents; terminal cells (39.5–)39.9–47.3–70.3(–71) × (10–)10.2–14.9–20(–20.2) μm, thin to thick-walled, cylindrical or clavate with obtuse apex; hyaline. Clamp connections present in all tissues.

Habitat

In small groups or occasionally solitary, on the ground near *Vateria indica* L., *Hopea parviflora* Bedd. and *Hopea ponga* (Dennst.) Mabb. (Dipterocarpaceae) and *Calophyllum* L. (Calophyllaceae J.Agardh), and *Myristica* Gronov. (Myristicaceae R.Br.) trees in India; on forest soil in tropical forest dominated by Dipterocarpaceae (*Dipterocarpus* spp., *Shorea* spp.) with some Fagaceae (*Quercus* spp., *Lithocarpus* spp. and *Castanopsis calathiformis*) in Thailand.

Known distribution

Known from India and Chiang Rai Province, northern Thailand.

Notes

Matheny *et al.* 2019 raised the status of *I.* sect. *Rimosae* (Fr.) Quél. to the genus *Pseudosperma* based on a six gene phylogenetic analysis. Species of *Pseudosperma* are probably widely distributed in Southeast Asia. *Pseudosperma keralense* can be recognized by its yellowish-brown pileus; lamellae with whitish, serrate edges; smooth, ellipsoidal basidiospores; a duplex pileipellis with superficial hyphae devoid of encrustations and encrusted hyphae beneath. The macro- and micro-morphological characters of the specimens from Thailand are similar to *P. keralense* reported from India. The combined phylogenetic analyses indicate the sequences of the Thai specimen *OR1629* cluster with the holotype of *P. keralense* from India with BS = 100%, PP = 1.0.

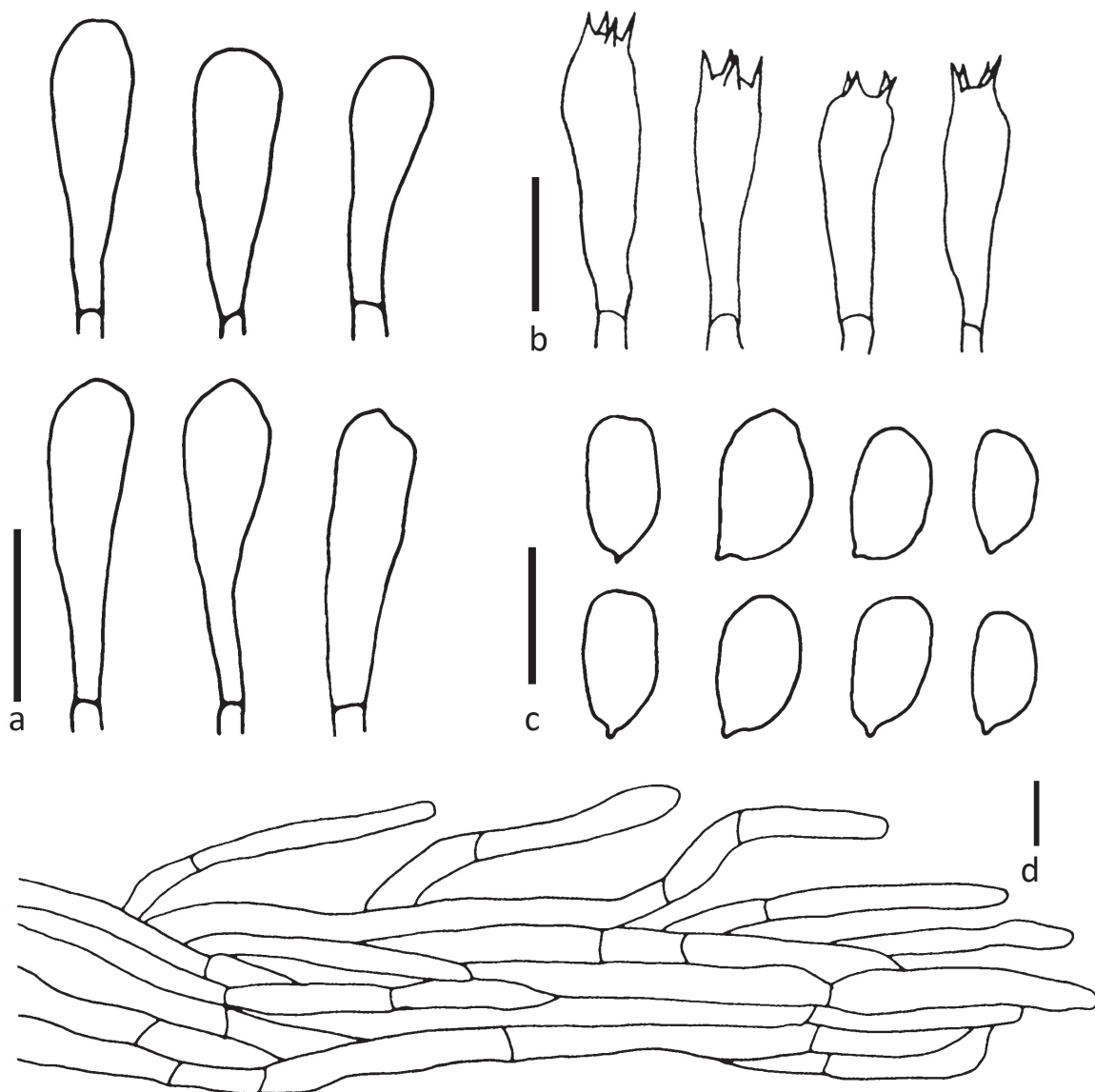


Fig. 5. Microscopic features of *Pseudosperma keralense* (K.P.D.Latha & Manim.) Matheny & Esteve-Rav. **a.** Cheilocystidia. **b.** Basidia. **c.** Basidiospores. **d.** Pileipellis. Scale bars: a–b, d, = 20 µm, c = 10 µm.

Discussion

Matheny *et al.* (2019), showed that the Inocybaceae was taxonomically and genetically very diverse, which led to a revised classification with the introduction of additional genera. Additional studies have continued to reveal new taxa of Inocybaceae worldwide (Matheny & Kudzma 2019; Bandini *et al.* 2021; Li *et al.* 2021; Khan *et al.* 2022). During our survey of Basidiomycetes in tropical forests of northern Thailand, we discovered a new species of *Inocybe*, *I. hopeae* sp. nov., and recorded the presence of *Pseudosperma keralense* for the first time in Thailand.

Both morphological characters and phylogenetic analyses identified *I. hopeae* sp. nov. as a close relative to *I. thailandica*, which was also described from tropical forests of northern Thailand by Horak *et al.* (2015). However, despite the similarity in appearance, *I. hopeae* is distinct from *I. thailandica* by having bigger basidiomes, different basidiome colour, longer basidia and longer cheilocystidia. Furthermore, *I. hopeae* has shorter pleurocystidia as compared to its cheilocystidia, which is not the case in *I. thailandica*. The spore size of *I. hopeae* have a bimodal distribution (Fig. 6) and is more variable than in *Inocybe thailandica*. Bimodal spore size distribution is usually indicative of the presence of both 2-spored and 4-spored basidia, but interestingly no 2-spored basidia were observed during the micro-morphological examination of the specimens.

Inocybe is a significant component of ectomycorrhizal communities in temperate, boreal and tropical forests (Matheny *et al.* 2009; Kokkonen & Vauras 2012). The habitat of *I. hopeae* sp. nov. recalls that of several species from the tropical highlands and lowlands of Southeast Asia. *Inocybe hopeae* was found to be putatively growing in association with Dipterocarpaceae and Fagaceae, similar to many Southeast Asian species like *I. hydrocybiformis* Horak, *I. stellata* Horak, Matheny & Desjardin and *I. thailandica* Horak, Matheny & Desjardin. Nevertheless, in addition to being found around trees of *Dipterocarpus* C.F.Gaertn., *Quercus* L. and *Castanopsis* (D.Don) Spach, *I. hopeae* was also found growing in association with *Hopea odorata*, which was not the case for the other species.

Over the past decade, only a single study (Horak *et al.* 2015) documented the diversity of *Inocybe* in Thailand, as compared to the other Southeast Asian regions from which several new species of *Inocybe* have been described (Yu *et al.* 2020; Li *et al.* 2021). With the high diversity of ectomycorrhizal Basidiomycetes in northern Thailand and their limited documentation (Horak *et al.* 2015; Thongbai *et al.* 2018; Vadthanarat *et al.* 2021), further studies may yet uncover additional new species of Inocybaceae.

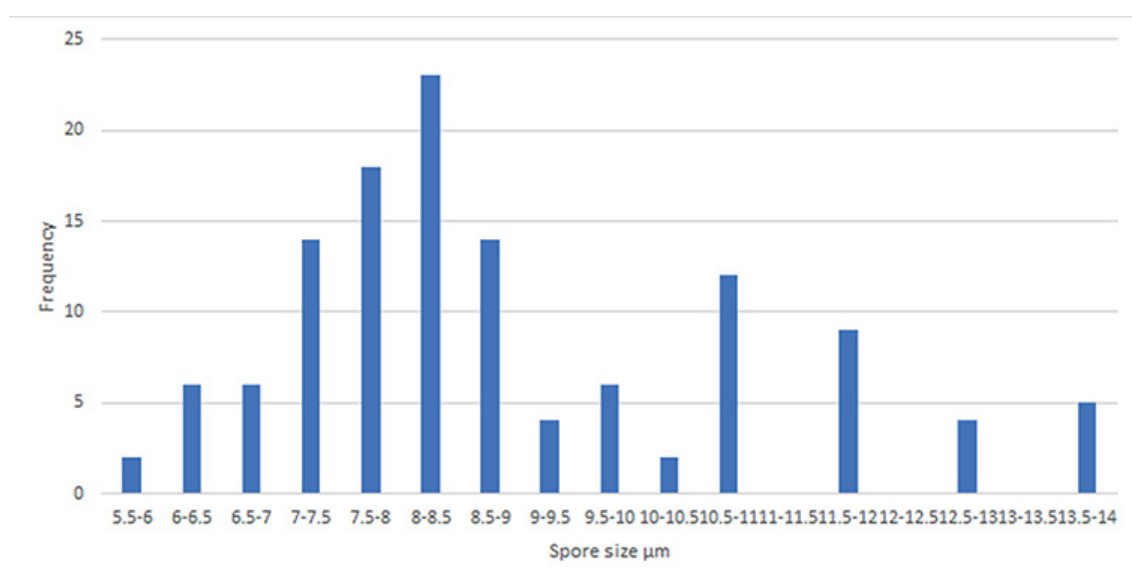


Fig. 6. Frequency distribution of spore size for *Inocybe hopeae* Raghoonundon & Raspé sp. nov.

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Author contributions

Conceived and designed study, O.R. and B.R.; analyzed data, B.R. and O.R.; collected specimens, B.R. and O.R.; performed experiments, B.R. and Y.H.; wrote the manuscript, B.R., S.V. and O.R.; reviewed and edited the manuscript, all authors. All authors have read and agreed to the version of the manuscript.

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