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Discovery of a new species of the hitherto monotypic genus *Nothocybe* Matheny & K.P.D. Latha from tropical India

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ABSTRACT
A new species of *Nothocybe* Matheny & K.P.D. Latha, *N. vanadurgae* Manim. & K.P.D. Latha, sp. nov., is described from Kerala State, India, based on morphology and molecular phylogeny. The diagnostic characters of this species include small basidiocarps with a fibrillose-squamulose pileus, a fibrillose-pruinose stipe with a marginate-bulbous base, smooth basidiospores occasionally with a slight angularity, a sterile lamella-edge with cheilocystidia occasionally with a resinous substance at the apex and copious caulocystidia with a resinous coating at the apex. A phylogenetic analysis based on a concatenated dataset of nrITS, nrLSU and *rpb*2 confirmed the taxonomic position of this species within the genus *Nothocybe* of the family Inocybaceae. A comprehensive description of this species, photographs and line drawings of the basidiocarps and the microscopic structures, a detailed discussion and a phylogram showing its position within the genus *Nothocybe*, are provided.

KEY WORDS
Agaricales, Basidiomycota, phylogeny, taxonomy, tropical agarics, new species.

RÉSUMÉ
Découverte d'une nouvelle espèce du genre jusqu'alors monotypique Nothocybe *Matheny & K.P.D. Latha de l'Inde tropicale.*
Une nouvelle espèce de *Nothocybe* Matheny & K.P.D. Latha, *N. vanadurgae* Manim. & K.P.D. Latha, sp. nov., est décrite dans l'État du Kerala, en Inde, d'après sa morphologie et sa phylogénie moléculaire. Les caractères diagnostiques de cette espèce comprennent de petits basidiocarpes avec un chapeau fibrilleux-squamuleux, un stipe fibrilleux-pruineux à base marginée-bulbeuse, des basidiospores lisses parfois légèrement anguleuses, un bord de lamelle stérile avec des chéilocystides parfois résineuses à l'apex et de nombreuses caulocystides recouvertes d'un revêtement résineux à l'apex. Une analyse phylogénétique basée sur un ensemble de données concaténées de nrITS, nrLSU et *rpb*2 a confirmé la position taxonomique de cette espèce au sein du genre *Nothocybe* de la famille des Inocybaceae. Une description complète de cette espèce, des photographies et des dessins des basidiocarpes et des structures microscopiques, une discussion détaillée et un phylogramme indiquant sa position au sein du genre *Nothocybe* sont fournis.

MOTS CLÉS
Agaricales, Basidiomycota, phylogénie, taxonomie, agarics tropicaux, espèce nouvelle.

INTRODUCTION

Nothocybe Matheny & K.P.D. Latha is a recently described and as yet monotypic genus in the agaric family Inocybaceae (Matheny *et al.* 2020). Earlier, this genus was considered as an enigmatic lineage named Nothocybe, along with six other clades (*Inocybe sensu stricto*, Pseudosperma, Mallocybe, Inosperma, Auritella and Mallocybella), within the genus *Inocybe sensu lato*. This lineage was identified based on an unpublished collection of *Inocybe* (*Inocybe* sp. ZT9250) from Kerala State in southern India (Matheny 2009). Subsequently, Latha *et al.* (2016) described a noteworthy species of *Inocybe I. distincta* K.P.D. Latha & Manim. from Kerala State, which appeared identical to *Inocybe* sp. ZT9250, the only representative of the Nothocybe lineage. Matheny *et al.* (2020), in their study on Inocybaceae using multigene phylogeny, elevated the Nothocybe lineage to generic rank based on *I. distincta.* Their study also elevated three other clades, Inosperma, Pseudosperma and Mallocybe as genera. Thus, the family Inocybaceae presently accommodates *Inocybe* (Fr.) Fr. *sensu stricto*, *Inosperma* (Kühner) Matheny & Esteve-Rav., *Nothocybe*, *Pseudosperma* Matheny & Esteve-Rav. and *Mallocybe* (Kuyper) Matheny, Vizzini & Esteve-Rav. as well as the other two previously recognised genera, *Auritella* Matheny & Bougher and *Tubariomyces* Esteve-Rav. & Matheny (Matheny *et al.* 2020). The genus *Nothocybe* is phylogenetically sister to the genus *Inocybe sensu stricto* (Matheny *et al.* 2020).

The genus *Nothocybe* is characterised by a squamulose-rimulose pileus, a fibrillose-pruinose stipe; phaseoliform to ovate-elliptic basidiospores with an occasional weak angular outline, septate cheilocystidia covered apically with a resinous substance, a hymenium devoid of pleurocystidia, a cutis-type stipitipellis with copious caulocystidia formed as modified terminal cells of the stipitipellis hyphae at the stipe apex and a probable ectomycorrhizal association with *Acacia* trees (Latha *et al.* 2016; Matheny *et al.* 2020).

Studies on the inocyboid fungi of Kerala have so far discovered a total of 51 species including one species each from the genera *Auritella* and *Nothocybe,* eight from the genus *Inosperma,* ten from the genus *Pseudosperma* and 31 from the genus *Inocybe sensu stricto* (Matheny *et al.* 2012; Latha & Manimohan 2017; Latha *et al.* 2023; Crous *et al.* 2024). The other two genera, *Mallocybe* and *Tubariomyces* have no species representation so far from Kerala (Latha & Manimohan 2017).

During our continuing investigations on the floristics and molecular systematics of the family Inocybaceae of Kerala, we came across a remarkable inocyboid species that was found to be distinct from all other previously described species of this group. The morphological – as well as molecular phylogenetic analysis confirmed that the species belongs to the genus *Nothocybe*. This species is formally described and discussed here as a new species of *Nothocybe* that forms the second known species of this genus.

MATERIAL AND METHODS

Morphological studies

The basidiocarps of the new species were gathered from a sacred grove around the Poyilkavu Temple in Koyilandi, Kozhikode District, Kerala, India. This sacred grove is sandwiched between a sandy beach on the shore of the Arabian Sea and a National Highway and is spread across about 12 acres of sandy soil. It is densely covered with evergreen trees, lianas and undergrowth. The major vegetation in the sacred grove is *Vatica chinensis* L.*, Abrus pulchellus* Wall.*, Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC.*, Lygodium scandens* (L.) SW.*, Phyllanthus reticulatus* Poir., *Scleropyrum pentandrum* (Dennst.) Mabb.*, Strychnos minor* Dennst.*, Leea indica* (Burm.f.) Merr. and *Nothopegia travancorica* Bedd. ex Hook.fil.. *Vatica chinensis* (Dipterocarpaceae) represents the most common and dominant tree in the grove. Conventional taxonomy as well as molecular phylogenetic studies were employed in this study. The macro-morphological features were recorded based on fresh collections. The colour names and the corresponding alphanumeric colour codes were recorded following Kornerup & Wanscher (1978) (e.g., 6C4) and the Online Auction Color Chart (Anonymous 2004) (e.g., OAC729). To observe the microscopic structures, thin sections were made from different parts of the basidiocarp and stained with a mixture of 1% aqueous solutions of both Congo red and phloxine and mounted in 3% KOH. The colours of the microscopic structures were recorded using sections mounted in distilled water. For evaluation of the range of basidiospore size, twenty basidiospores of each basidiocarp were measured for length and width. The mean and the standard deviation for both the length and the width along with the range of spore quotient (Q, length/width ratio) and its mean value (Qm) were calculated. The measurement of basidiospore length does not include the hilar appendix. The holotype of the new species is deposited at the Calicut University Herbarium (CALI) of the Department of Botany, University of Calicut, Kerala, India. Scanning electron micrographs were obtained using a JEOL JSM-6390 SEM at an accelerating voltage of 10 kV, following the protocol described in Latha *et al.* (2016).

DNA extraction, amplification and sequencing

The DNA was isolated from dried basidiocarps of the new species using a NucleoSpin Plant II DNA extraction kit (Macherey-Nagel, Düren, Germany) based on the protocol recommended for fungi in the manual of the kit. Three gene regions, the nuclear ribosomal internal transcribed spacer region (nrITS), the nuclear ribosomal large subunit (nrLSU) and a the nuclear second-largest subunit of RNA polymerase II (*rpb*2) were analysed in this study. The primer pairs ITS1/ITS4 (White *et al.* 1990), LR0R/LR7 (Vilgalys & Hester 1990) and b6f/b7.1R (Matheny 2005) were used for amplifying the nrITS, nrLSU and *rpb*2 genes respectively. The amplification reactions were conducted in a Bio-Rad C1000 Thermal Cycler (United States). The cycling parameters of PCR described in Latha & Manimohan (2015) were followed for amplification of the gene regions. The amplification of PCR products was analysed with agarose gel electrophoresis. The confirmed PCR products were subsequently purified using the GeneJet™ PCR Purification Kit (Thermo Fisher Scientific, Mumbai, India), following the manufacturer's protocol. The purified products were then subjected to automated DNA sequencing

at a commercial facility using an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, United States) using the same primers used for PCR reactions. The newly generated sequences were quality-checked and trimmed using the software Sequencher V4.10.1 (Gene Codes Corporation, Ann Arbor MI United States). The verified sequences have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Taxon sampling, sequence alignment and phylogenetic analysis

The newly generated sequences (nrITS, nrLSU and *rpb*2) were used for the phylogenetic analysis. All the new sequences were subjected to BLASTn searches to find out the related species for which sequences were available in the NCBI's GenBank database. A concatenated phylogenetic dataset (nrITS + nrLSU + *rpb*2) was prepared to run the analysis. The dataset comprised nrITS, nrLSU and *rpb*2 sequences of the new species generated during this study along with the sequences from the BLASTn search results and the sequences representing the species of the seven genera (*Inocybe, Nothocybe, Pseudosperma, Inosperma, Auritella, Tubariomyces* and *Mallocybe*) of Inocybaceae. In addition, sequences of six species of Crepidotaceae (*Crepidotus prostratus* Cleland*, Crepidotus applanatus* (Pers.) P.Kumm.*, Pleuroflammula flammea* (Murrill) Singer *P. praestans* E.Horak*, Simocybe phlebophora* E.Horak and *S. serrulata* (Murill) Singer) were also incorporated as outgroups following Matheny *et al.* (2020). The dataset included a total of 102 sequences of three gene regions from 97 species including the outgroup taxa. The sequences in the dataset were aligned independently (nrITS, nrLSU and *rpb*2) using the MAFFT web tool (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) with default settings. The aligned datasets were then manually refined using AliView v.1.27 (Larsson 2014). Following alignment and trimming, the nrITS, nrLSU and *rpb*2 sequence datasets were concatenated using SeaView v.4.7 (Gouy *et al.* 2010). The concatenated dataset encompassed a total of 2153 characters, including gaps, with 540 characters for nrITS, 925 for nrLSU and 688 for *rpb*2. The details of the taxa used for the phylogenetic analysis are listed in Table 1. The phylogenetic analysis was conducted using Maximum Likelihood (ML) in RAxML-HPC2 (v.8.2.10) (Stamatakis 2014) on the XSEDE platform as implemented in the CIPRES Science Gateway web portal (Miller *et al.* 2010). RAxML was run using the evolutionary model, GTRCAT, as recommended in the RAxML user manual, with 1000 rapid ML bootstrap replicates and the default settings for other options. The phylogram generated from the ML analysis was visualised using FigTree v.1.4.3 (Rambaut 2014). The aligned sequence dataset has been deposited in Figshare.

RESULTS

Phylogenetic results

The ML analysis, based on a combined dataset of nrITS, nrLSU and *rpb*2 sequences, resulted in a phylogram that is presented in Fig. 1. The phylogram recovered a large monophyletic group representing the family Inocybaceae with full (100 BS) bootstrap support. Within this large monophyletic group, seven distinct clades representing the seven genera (*Inocybe sensu stricto, Nothocybe, Pseudosperma, Inosperma, Tubariomyces, Auritella* and *Mallocybe*) of the family Inocybaceae were identified. The new species nested within the clade representing the genus *Nothocybe.* Within this clade, the new species was found to be a lineage sister to the only known species of the genus *Nothocybe N. distincta,* with significant bootstrap (73% BS) support. The clade representing the genus *Nothocybe* appeared as a lineage sister to *Inocybe sensu stricto* with significant bootstrap (88% BS) support.

TAXONOMY

Family Inocybaceae Jülich
Genus *Inocybe* (Fr.) Fr.

Nothocybe vanadurgae Manim. & K.P.D. Latha, sp. nov.
(Figs 2; 3; 4;)

Type material. — **India** • Kerala State, Kozhikode District, the sacred grove around Poyilkavu Temple; on soil; near *Vatica chinensis* (Dipterocarpaceae) trees; 11°24'31.6"N, 75°42'48.6"E; 11.XI.2022; *P. Haridev DKP-SERB104*; holoype: CALI[CALI DKP-SERB104); GenBank no. nrITS: (DKP-SERB104: PV050412); nrLSU (DKP-SERB104: PV050413); and *rpb*2 (DKP-SERB104: PV155511).

MycoBank number. — MB 857480.

Systematic position. — Basidiomycota, Agaricomycetes, Agaricales, Inocybaceae, *Nothocybe*.

Etymology. — Named after Vanadurga (literally, in Sanskrit, goddess of the forest), the deity of Poyilkavu Temple, as this species was collected from the sacred grove around this temple.

Diagnosis. — Basidiocarps small. Pileus brownish orange with a fibrillose-squamulose surface. Lamellae adnexed to narrowly adnate, white to orange grey, with a fimbriate, concolourous edge. Stipe central, equal or slightly tapering towards the apex, solid, fibrillose, with a pruinose surface. Basidiospores ovoid to obovoid or amygdaliform, smooth, occasionally with a slight angular outline. Pleurocystidia absent. Cheilocystidia copious, occasionally with a resinous coating at the apex. Pileipellis a cutis disrupted by patches of ascending hyphae. Caulocystidia as modified terminal hyphae of stipitipellis, coated with a resinous substance. Differs from *N. distincta* in having a paraboloid to convex pileus with a straight margin, adnexed or narrowly adnate lamellae, a white stipe with a marginate-bulbous base, ovoid to obovoid or amygdaliform basidiospores, slightly larger caulocystidia and distinct nrITS, nrLSU and *rpb*2 sequences.

Habitat and distribution. — On the ground, near *Vatica chinensis* (Dipterocarpaceae) trees, solitary or in small groups. Known only from the type locality in Kerala State, India.

Description

Basidiocarps
Small.

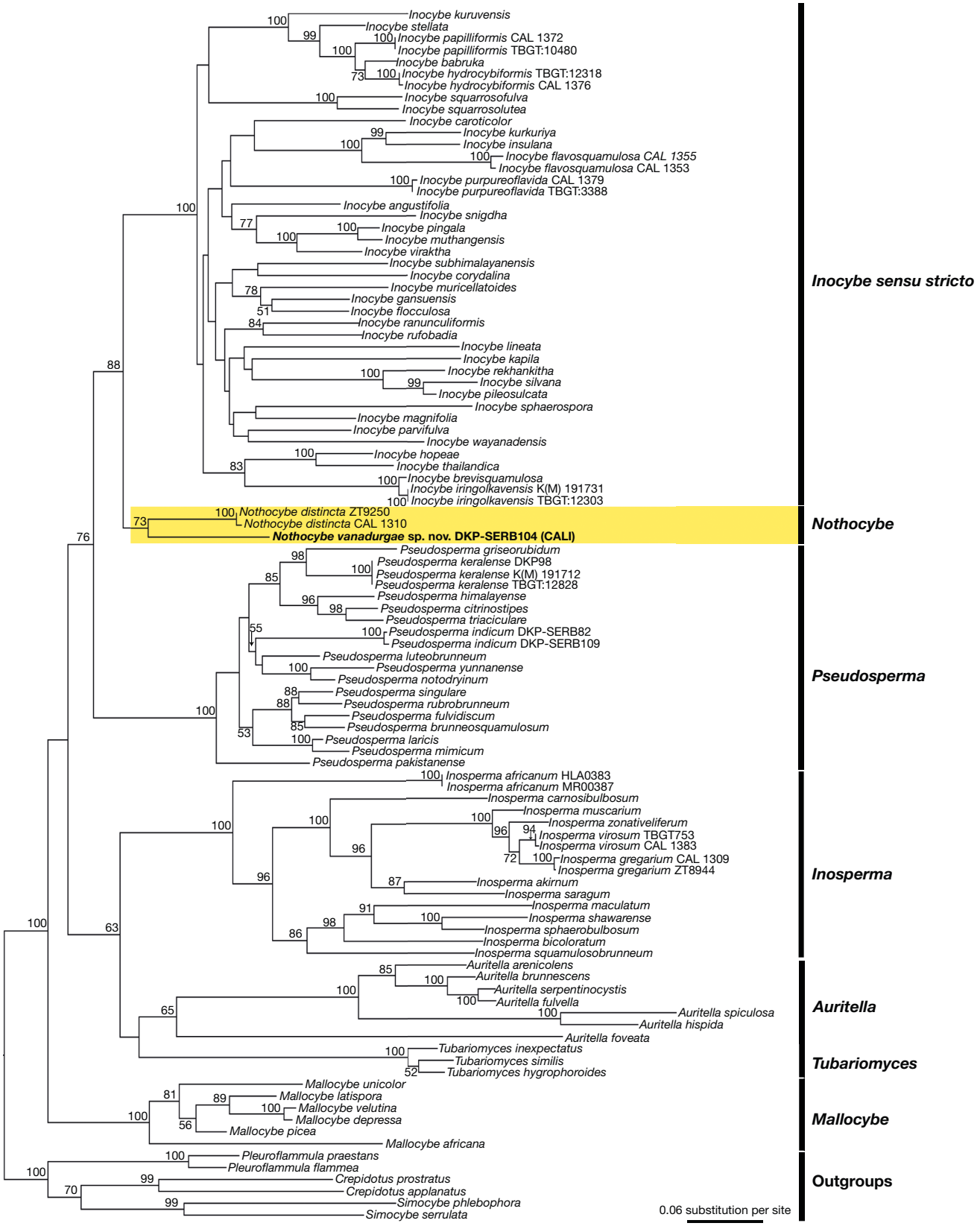

Fig. 1. — ML phylogram illustrating the placement of *Nothocybe vanadurgae* Manim. & K.P.D. Latha, sp. nov. within the genus *Nothocybe*, inferred from a concatenated dataset of nrITS, nrLSU and *rpb2* sequences of the family Inocybaceae. The new species is highlighted in **bold font** and species representing the genus *Nothocybe* Matheny & K.P.D. Latha are marked with a **yellow background**. Support values at the nodes represent maximum likelihood bootstrap (mlb) percentages, with values ≥50% displayed.

Fig. 2. — *Nothocybe vanadurgae* Manim. & K.P.D. Latha, sp. nov.: DKP-SERB104 (CALI, holotype): **A**, basidiocarps in natural habitat; **B**, basidiospores; **C**, basidium; **D**, cheilocystidia; **E**, pileipellis; **F**, stipitipellis; **G**, caulocystidia. Scale bars: A, 10 mm; B-D G, 10 µm; E, 50 µm; F, 20 µm. Photos credits: by P. Haridev.

TABLE 1. — Details of the taxa used in the phylogenetic analysis. Strain/voucher number, their geographical location and the GenBank accession numbers of their nrITS, nrLSU and *rpb2* sequences are listed. The details of the new species are highlighted in **bold.**

Taxa	Strain/Voucher Number	nrITS	nrLSU	*rpb2*	Locality
Auritella arenicolens (Cleland) Matheny & Bougher	PERTH:08047081	KJ729857	KJ702338	KJ729920	Australia
Auritella brunnescens Matheny & Bougher	NLB942	KJ702342	KJ702339	KJ702347	Australia
Auritella foveata C.K.Pradeep & Matheny	TBGT9631	GU062740	GU062739	GU062738	India
Auritella fulvella Matheny & Bougher	BRI:AQ669485	KJ702355	KJ70235	KJ702357	Australia
Auritella hispida Matheny & T.W.Henkel	TH9857	KT378202	KT378208	KT378213	Cameroon
Auritella serpentinocystis Matheny, Trappe & Bougher ex Matheny & Bougher	TENN:063641	KJ729858	JQ313559	KJ756402	Australia
Auritella spiculosa Matheny & T.W.Henkel	TH9866	KT378204	KT378206	KT378214	Cameroon
Crepidotus prostratus Cleland	PBM3463	HQ728537	HQ728538	HQ728540	Australia
Crepidotus applanatus (Pers.) P.Kumm.	PBM717	DQ202273	AY380406	AY333311	United States
Inocybe angustifolia (Corner & E.Horak) Garrido	DED8139	GQ892988	GQ892942	MH577422	Thailand
Inocybe babruka K.P.D.Latha & Manim.	CAL 1344	KY440086	KY549116	KY553237	India
Inocybe brevisquamulosa E.Horak, Matheny & Desjardin	ZT10102 (SFSU)	GQ893019	GQ892974	—	Thailand
Inocybe caroticolor T.Bau & Y.G.Fan	AST47	MH473148	MH536985	—	Pakistan
Inocybe corydalina Quél.	EIU:AM10687	MH216083	MH220259	MH249790	Russia
Inocybe flavosquamulosa C.K.Pradeep & Matheny	CAL 1353	KY440087	KY549117	KY553238	India
Inocybe flavosquamulosa C.K.Pradeep & Matheny	CAL 1355	KY440088	KY549118	—	India
Inocybe flocculosa Sacc.	ZRL20151789	LT716045	KY418861	KY419007	China
Inocybe gansuensis T.Bau & Y.G.Fan	2012150	KY402221	KY402217	KY402219	China
Inocybe hopeae Raghoonundon & Raspe, 2023	OR1665	—	ON831503	ON553692	Thailand
Inocybe hydrocybiformis (Corner & E.Horak) Garrido	CAL1376	KY440090	KY549120	KY553240	India
Inocybe hydrocybiformis (Corner & E.Horak) Garrido	TBGT:12318	KP171130	KP170911	—	India
Inocybe insulana K.P.D.Latha & Manim.	CAL 1258	KY440092	KY549122	KY553241	India
Inocybe iringolkavensis K.P.D.Latha & Manim.	K(M) 191731	KM924524	KM924519	KY553242	India
Inocybe iringolkavensis K.P.D.Latha & Manim.	TBGT:12303	KT329447	KT329453	KT329442	India
Inocybe kapila K.P.D.Latha & Manim.	CAL 1346	KY440093	KY549123	—	India
Inocybe kurkuriya K.P.D.Latha & Manim.	CAL 1352	KY440095	KY549125	KY553245	India
Inocybe kuruvensis K.P.D.Latha & Manim.	K(M) 191734	KM924522	KM924517	KY553246	India
Inocybe lineata E.Horak, Matheny & Desjardin	DED8048	—	GQ892958	KM245999	Thailand
Inocybe magnifolia Matheny, Aime & T.W.Henkel	MCA 2441	JN642228	JN642244	EU600899	Guyana
Inocybe muricellatoides T.Bau & Y.G.Fan	2012130	KY402220	KY402216	KY402218	China
Inocybe muthangensis K.P.D.Latha & Manim.	K(M) 191735	KM924521	KM924516	KY553247	India
Inocybe papilliformis C.K.Pradeep & Matheny	TBGT:10480	KP171131	KP170912	KM245988	India
Inocybe papilliformis C.K.Pradeep & Matheny	CAL 1372	KY440096	KY549126	—	India
Inocybe parvifulva Matheny & Bougher	TENN:066967	—	KP170975	KM406214	Australia
Inocybe pileosulcata E.Horak, Matheny & Desjardin	CAL 1362	KY440098	KY549128	KT329445	India
Inocybe pingala K.P.D.Latha & Manim.	CAL 1345	KY440100	KY549130	KY553248	India
Inocybe purpureoflavida K.B.Vrinda & C.K.Pradeep	CAL 1379	KY440101	KY549131	—	India
Inocybe purpureoflavida K.B.Vrinda & C.K.Pradeep	TBGT:3388	KT329451	KT329457	KT329445	India
Inocybe ranunculiformis Sand.-Leiva, Caiafa & M.E.Sm.	PSL-1589	MT367472	MT372890	MT374799	Chile
Inocybe rekhankitha K.P.D.Latha & Manim.	CAL 1356	KY440102	KY549132	—	India
Inocybe rufobadia Matheny & Bougher	PERTH:08320454	KF977213	KF915290	KF991385	Australia
Inocybe silvana K.P.D.Latha & Manim.	CAL 1259	KY440104	KY549134	—	India
Inocybe snigdha K.P.D.Latha & Manim.	CAL 1350	KY440105	KY549135	KY553250	India
Inocybe sphaerospora Kobayasi	DED8059 (SFSU)	GQ892993	GQ892948	MH577472	Thailand
Inocybe squarrosofulva S.N.Li, Y.G.Fan & Z.H.Chen	MHHNU31927	MZ050802	MW715815	MW729766	China
Inocybe squarrosolutea (Corner & E.Horak) Garrido	MHHNU8984	MK388162	MW709446	MW715636	China
Inocybe stellata E.Horak, Matheny & Desjardin	CAL 1369	KY440106	KY549136	KY553251	India
Inocybe subhimalayanensis Razzaq, Naseer & Khalid	*Inocybe*_sp_5	ON810647	—	—	Pakistan
Inocybe thailandica E.Horak, Matheny & Desjardin	SFSU:DED8049	GQ893013	GQ892968	KM656129	Thailand
Inocybe viraktha K.P.D.Latha & Manim.	CAL 1357	KY440107	KY549137	KY553252	India
Inocybe wayanadensis K.P.D.Latha & Manim.	K(M) 191737	KM924520	KM924515	KY553254	India
Inosperma africanum Aïgnon, Yorou & Ryberg	HLA0383	MT534298	MT560733	—	Benin
Inosperma africanum Aïgnon, Yorou & Ryberg	MR00387	MN096189	MN097881	MT770739	Togo
Inosperma akirnum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL1358	KY440085	KY549115	KY553236	India
Inosperma bicoloratum (E.Horak, Matheny & Desjardin) Matheny & Esteve-Rav.	ZT12187 (SFSU)	GQ892984	GQ892938	JQ846464	Malaysia
Inosperma carnosibulbosum (C.K.Pradeep & Matheny) Matheny & Esteve-Rav.	TBGT:12047	KT329448	KT329454	KT329443	India
Inosperma gregarium (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL1309	KX852305	KX852306	KX852307	India
Inosperma gregarium (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	ZT 8944	—	EU600903	EU600902	India
Inosperma maculatum (Boud.) Matheny & Esteve-Rav.	EL12604	AM882964	AM882964	—	Sweden

Appendix 1. — Continuation.

Taxa	Strain/Voucher Number	nrITS	nrLSU	*rpb2*	Locality
Inosperma muscarium Y.G.Fan, L.S.Deng, W.J.Yu & N.K.Zeng	FYG6091	MZ373982	MZ373991	MZ388093	China
Inosperma saragum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL1360	KY440103	KY549133	KY553249	India
Inosperma shawarense (Naseer & Khalid) Aïgnon & Naseer	FLAS-F-S9456	KY616964	KY616966	–	Pakistan
Inosperma sphaerobulbosum S.N.Li, Y.G.Fan & Z.H.Chen	MHHNU32266	OP135501	OP134001	OP161559	China
Inosperma squamulosobrunneum S.N.Li, Y.G.Fan & Z.H.Chen	MHHNU32359	OP135499	OP134000	OP161562	China
Inosperma virosum (K.B.Vrinda, C.K.Pradeep, A.V.Joseph & T.K.Abraham ex C.K.Pradeep, K.B.Vrinda & Matheny) Matheny & Esteve-Rav.	TBGT753	KT329452	KT329458	KT329446	India
Inosperma virosum (K.B.Vrinda, C.K.Pradeep, A.V.Joseph & T.K.Abraham ex C.K.Pradeep, K.B.Vrinda & Matheny) Matheny & Esteve-Rav.	CAL 1383	KY440108	KY549138	KY553253	India
Inosperma zonativeliferum Y.G.Fan, H.J.Li, F.Xu, L.S.Deng & W.J.Yu	FYG6441	OL850878	OM845772	ON075044	China
Mallocybe africana Aïgnon, Yorou & Ryberg	MR00385	MN096194	MN097886	MT465593	Togo
Mallocybe depressa L. Fan, H. Zhou & N. Mao	BJTC FM1695	OM801899	OM801904	OM780100	China
Mallocybe latispora (Bon) Matheny & Esteve-Rav.	JV19640F	MN178503	MN178529	MN203520	Finland
Mallocybe picea L. Fan & N. Mao	BJTC FM555	OM801896	OM801901	OM780096	China
Mallocybe unicolor (Peck) Matheny & Esteve-Rav.	PBM2974	MN178524	JQ313569	MN203532	United States
Mallocybe velutina Saba & Khalid	MSM # 0048	MK990129	MK999927	–	Pakistan
***Nothocybe distincta* (K.P.D.Latha & Manim.) Matheny & K.P.D.Latha**	**CAL 1310**	**KX171343**	**KX171344**	**KX171345**	**India**
Nothocybe distincta (K.P.D.Latha & Manim.) Matheny & K.P.D.Latha	ZT9250	–	EU604546	EU600904	India
***Nothocybe vanadurgae* Manim. & K.P.D. Latha, sp. nov.**	**DKP-SERB104 (CALI)**	**PV050412**	**PV050413**	**PV155511**	**India**
Pleuroflammula flammea (Murrill) Singer	MCA339	DQ494685	AF367962	DQ474124	Virginia
Pleuroflammula praestans E.Horak	PBM3461	HQ832450	HQ832464	HQ832441	Australia
Pseudosperma brunneosquamulosum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL 1308	KX073582	KX073586	KX073589	India
Pseudosperma citrinostipes Y.G.Fan & W.J.Yu	FYG2903	MT072897	MT071202	–	China
Pseudosperma fulvidiscum Y.G.Fan, L.N.Zhao & W.J.Yu	FYG6067	OM135594	OM350000	OM780119	China
Pseudosperma griseorubidum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL:1253	KT180326	KT180327	KT180328	India
Pseudosperma himalayense (Razaq, Khalid & Takah. Kobay.) Matheny & Esteve-Rav.	K7	MH745138	–	–	Pakistan
Pseudosperma indicum K.P.D. Latha, Haridev & Manim.	DKP-SERB109	OR243200	OR243199	OR253891	India
Pseudosperma indicum K.P.D. Latha, Haridev & Manim.	DKP-SERB82	OR243198	OR243197	OR253892	India
Pseudosperma keralense (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	DKP98	KY440094	KY549124	KY553244	India
Pseudosperma keralense (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	TBGT:12828	KP636861	KP171060	KM656099	India
Pseudosperma keralense (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	K(M) 191712	NR_160442	NG_064382	KY553243	India
Pseudosperma laricis L.Fan & N.Mao	BJTC FM887	OM801905	OM801912	–	China
Pseudosperma luteobrunneum (K.P.D.Latha & Manim.) Matheny & Esteve-Rav.	CAL 1260	KX073580	KX073584	KX073588	India
Pseudosperma mimicum (Massee) Matheny & Esteve-Rav.	MSM#008	KJ546158	–	–	Pakistan
Pseudosperma notodryinum (Singer, I.J.A.Aguiar & Ivory) Matheny & Esteve-Rav.	CSU 01252	MH578028	MK421970	MH577509	United States
Pseudosperma pakistanense (Z.Ullah, S.Jabeen, H.Ahmad & A.N.Khalid) Matheny & Esteve-Rav.	LAH35285	MF588965	–	–	Pakistan
Pseudosperma rubrobrunneum (K.P.D.Latha & Manim.) Y.G.Fan	CAL 1307	KX073583	KX073587	KX073590	India
Pseudosperma singulare Y.G.Fan, L.N.Zhao & W.J.Yu	FYG6339	OM135605	OM149380	OM780122	China
Pseudosperma triaciculare Saba & Khalid	MSM 0039	MG742423	MG742424	–	Pakistan
Pseudosperma yunnanense (T.Bau & Y.G.Fan) Matheny & Esteve-Rav.	HMJAU25840	MH047250	MG844975	–	China

Appendix 1. — Continuation.

Taxa	Strain/Voucher Number	nrITS	nrLSU	*rpb2*	Locality
Simocybe phlebophora E.Horak	PBM3089	MK421963	MK421967	MK415449	New Zealand
Simocybe serrulata (Murrill) Singer	PBM2536	DQ494696	AY745706	DQ484053	United States
Tubariomyces hygrophoroides Esteve-Rav., P.-A. Moreau & C.E.Hermos.	P05112008	GU907097	GU907094	GU907090	France
Tubariomyces inexpectatus (M.Villarreal, Esteve-Rav., Heykoop & E.Horak) Esteve-Rav. & Matheny	AH25500	GU907095	GU907091	GU907088	Spain
Tubariomyces similis Della Magg., Tolaini & Vizzini	RFS0805	GU907096	GU907092	GU907089	Spain

Pileus
8-23 mm diam., initially paraboloid, becoming hemispherical or convex; surface brownish orange (6C4/OAC688) all over when young, becoming brown or dark brown (6E8/6F8OAC734) at the centre and on the squamules and brownish orange (6C4/OAC729) elsewhere with age, initially appressed-fibrillose all over, becoming appressed – or slightly recurved-squamulose on and around the centre and appressed-fibrillose elsewhere at maturity; margin deflexed or somewhat straight when young, becoming straight at maturity, crenate.

Lamellae
Adnexed or narrowly adnate, close, initially white, becoming white to orange grey (5B2/OAC718) at maturity, up to 2 mm wide, with lamellulae in 1-2 tiers; edge fimbriate, white or concolourous with the sides.

Stipe
7-26 × 2-4 mm, central, terete, initially equal, becoming equal or slightly tapering towards the apex at maturity, cartilaginous, solid; surface white, appressed-fibrillose all over, pruinose all over, densely so towards the apex; base distinctly marginate-bulbous. Context soft, up to 2 mm wide, white.

Odour
Not distinctive.

Taste
Not distinctive.

Spore print
Not obtained.

Basidiospores
9-11(12) × (5)6-7(8) (10.5 ± 0.7 × 6.3 ± 0.6) µm, Q = 1.3-2.0 Qm = 1.6, smooth, ovoid or obovoid in frontal view, amygdaliform with an acute apex in side view, occasionally with a slight angularity, thick-walled, pale yellowish brown.

Basidia
32-40 × 10-13 µm, clavate or at times pedicellate clavate, thin-walled, hyaline, 4-spored; sterigmata up to 5 µm long.

Pleurocystidia
Absent.

Lamella-edge
Sterile with clusters of cheilocystidia.

Cheilocystidia
28-67 × 9-16 µm, abundant, cylindrical, clavate or utriform, occasionally coated with a resinous substance especially towards the apex, slightly thick-walled, hyaline.

Lamellar trama
Subregular; hyphae 4-12 µm wide, thin-walled, hyaline.

Pileus trama
Subregular; hyphae 5-16 µm wide, thin-walled, with a yellowish brown plasmatic pigment and occasional brown spiral encrustations.

Pileipellis
A cutis disrupted by patches of ascending hyphae; hyphae 3-8 µm wide, slightly thick-walled, with a yellowish brown wall pigment.

Stipitipellis
A cutis disrupted by bunches of caulocystidia over the entire length of the stipe; hyphae 4-12 µm wide, slightly thick-walled, hyaline or with a pale yellow wall pigment.

Caulocystidia
Occurring as modified terminal hyphal cells of stipitipellis, 21-100 × 8-16 µm, abundant, versiform: cylindrical, narrowly utriform, narrowly utriform with a median constriction, flexuous or cylindrico-flexuous, with a subcapitate, capitate or obtuse apex, occasionally septate, occasionally coated with a resinous substance especially towards the apex, slightly thick-walled, hyaline or with hyaline spiral encrustations.

Clamp connections
Observed on all hyphae.

Notes
Nothocybe vanadurgae Manim. & K.P.D. Latha, sp. nov. is characterised by small basidiocarps with a fibrillose-squamulose pileus, adnexed to narrowly adnate and white to orange grey lamellae, a fibrillose-pruinose stipe with a marginate-bulbous base, smooth basidiospores occasionally with a slight angularity, a sterile lamella-edge with abundant cheilocystidia

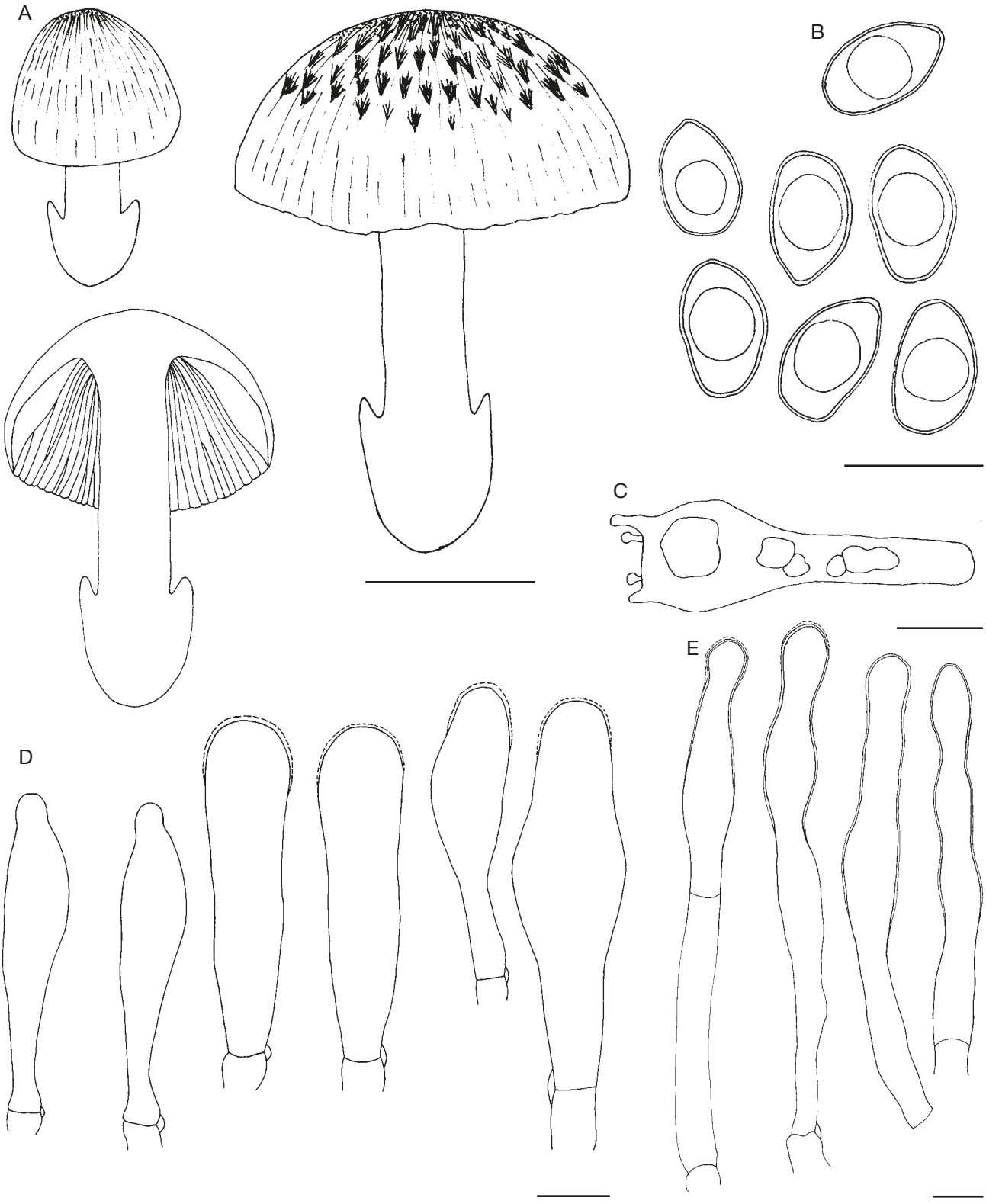

FIG. 3. — *Nothocybe vanadurgae* Manim. & K.P.D. Latha, sp. nov.: DKP-SERB104 (CALI, holotype): **A**, basidiocarps; **B**, basidiospores; **C**, basidium; **D**, cheilocystidia; **E**, caulocystidia. Scale bars: A, 10 mm; B-E, 10 µm. Drawings by P. Haridev & M. M. Manjulakshmi.

occasionally with a resinous substance at the apex; a cutis-type pileipellis disrupted by patches of ascending hyphae and bunches of caulocystidia scattered over the entire length of the stipitipellis formed as modified terminal hyphae, occasionally coated with a resinous substance and a probable association with *Vatica chinensis* (Dipterocarpaceae).

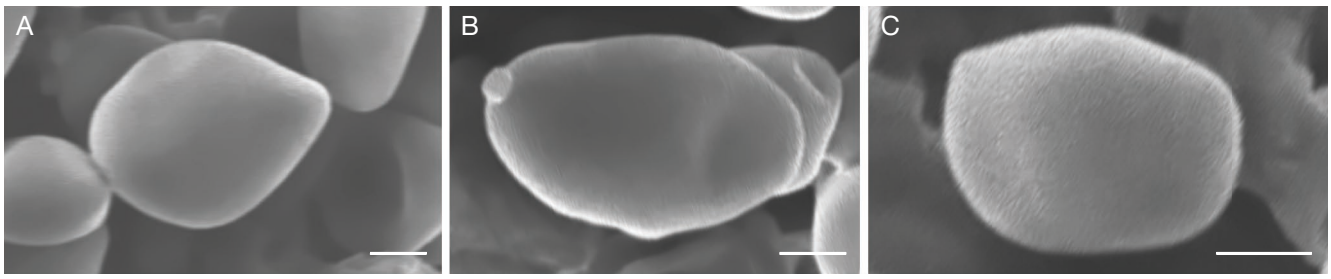

Fig. 4. — *Nothocybe vanadurgae* Manim. & K.P.D. Latha, sp. nov.: DKP-SERB104 (CALI, holotype): **A**-**C**, basidiospores under Scanning Electron Microscope (SEM). Scale bars: A-C, 2 µm.

Nothocybe distincta (K.P.D. Latha & Manim.) Matheny & K.P.D. Latha, a species originally described as *Inocybe distincta* K.P.D. Latha & Manim. from Kerala, India (Latha *et al.* 2016), represents the only other known species of the genus. *Nothocybe distincta* is similar to *N. vanadurgae* Manim. & K.P.D. Latha, sp. nov. in having almost similar size (pileus 6-17 mm; stipe 9-31 × 2-4 mm) and colour of the basidiocarps, a fibrillose-squamulose pileus, similar-coloured lamellae with a white edge, an appressed-fibrillose stipe with a pruinose surface, similar size of the basidiospores (6-12 × 5-7 µm) with an occasional slight angular outline, a sterile lamella-edge, similar size (17-70 × 7-10 µm) and morphology of cheilocystidia with a resinous substance at the apex, a cutis-type pileipellis disrupted by patches of ascending hyphae and a cutis-type stipitipellis with caulocystidia having a resinous coating at the apex. However *N. distincta* has an applanate, rimulose pileus with a reflexed margin, emarginate lamellae, an orange grey stipe with an abruptly ending base, phaseoliform-ellipsoid basidiospores and slightly smaller (17-70 × 7-10 µm) caulocystidia. Moreover *N. distincta* was collected from *Acacia* groves.

No close hits with zero e-values were obtained using the nrITS (649 bp) sequence in BLASTn searches. However *N. distincta* resulted as the closest hit using the nrLSU (1332 bp: GenBank EU604546; 97.5% identity) and the *rpb*2 (651 bp: GenBank EU600904; 89.2% identity) sequences.

DISCUSSION

Among the seven genera of the family Inocybaceae, *Nothocybe* was the only genus that remained monotypic so far. The discovery of a second species of the genus from the same geographical region is remarkable. The species was collected during the northeast monsoon season of 2022. However, all attempts to collect additional specimens of this species failed. Considering the significance of this species as the second known species of the as-yet monotypic genus *Nothocybe*, it is described as a new species based on a single collection. This collection was made from an area where *Vatica chinensis* trees are the most common and dominant component of the vegetation. This suggests a potential association of this species with the angiosperm family Dipterocarpaceae. The consistent association of *Inocybe* species with trees of the family Dipterocarpaceae in Kerala State has already been highlighted by Latha & Manimohan (2017).

The generic circumscription of *Nothocybe* was based solely on the characters of its single known species *N. distincta* (Matheny *et al.* 2020). However, the discovery of *N. vanadurgae* Manim. & K.P.D. Latha, sp. nov. necessitates an expansion of the diagnostic features of the genus. The key morphological features that differentiate *N. vanadurgae* Manim. & K.P.D. Latha, sp. nov. from *N. distincta* are the nature of the stipe base, the morphology of the basidiospores and a possible association with a member of the family Dipterocarpaceae. Accordingly, the generic circumscription of *Nothocybe* is modified as follows: pileus finely squamulose to rimulose; stipe fibrillose-pruinose with an abrupt or marginate-bulbous base; basidiospores ovoid, ovo-elliptic or obovoid to amygdaliform or phaseoliform with occasional weak angular outline; cheilocystidia septate or non-septate and apically covered with a resinous substance; caulocystidia present (at the stipe apex) as modified terminal cells of stipitipellis hyphae; pleurocystidia absent; probable ectomycorrhizal association with *Acacia* Mill. or *Vatica chinensis*.

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