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Amanita indovaginata, a new species from tropical Sal forest in India

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Macrofungal explorations conducted in several Sal (*Shorea robusta*)-dominated tropical forests in West Bengal and Jharkhand, India, unveiled a noteworthy and previously unknown member of *Amanita* sect. *Vaginatae*, here proposed as *Amanita indovaginata*. This newly discovered species is substantiated by both morphological characteristics and sequence data from three genes (ITS, LSU, and *tef* 1-α). A comprehensive morphological description, complemented by illustrations, is provided to enhance the understanding of this novel species.

Key words: *Amanitaceae*, *Basidiomycota*, 3-locus phylogram, *Shorea robusta*, *Vaginatae*.

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V rámci výzkumu makromycetů, uskutečněného v několika tropických lesích s převahou damarovníku (sál, *Shorea robusta*) v indických státech Džhárkhand a Západní Bengálsko, byl odhalen pozoruhodný a dosud neznámý zástupce muchomůrek ze sekce *Vaginatae*, pro který je zde navrženo jméno *Amanita indovaginata*. Identita nového druhu je podložena jak morfologickou charakteristikou, tak sekvenčními daty ze tří genů (ITS, LSU a *tef* 1-α). Pro jeho dobré poznání je uveden obsáhlý morfologický popis, doplněný ilustracemi stěžejních znaků.

Introduction

The genus *Amanita* is the most species-rich genus in the family *Amanitaceae*, distributed over all continents with the exception of Antarctica (Codjia et al. 2023). To date, 1000 *Amanita* species names have been proposed, out of which only ca 700 species are accepted globally (Cui et al. 2018, Yang et al. 2018, Kalichman et al. 2020). In India, approximately 66 different *Amanita* species are known to occur (Hosen et al. 2018, Mehmood et al. 2018, Kumar et al. 2022).

Recent investigations (Cui et al. 2018, Yang et al. 2018) divided this genus into three subgenera and eleven sections [subgenus *Amanita* Pers., containing sections *Amanita* Pers., *Amarrendiae* (Bougher et T. Lebel) Zhu L. Yang, Y.Y. Cui, Q. Cai et L.P. Tang, *Caesareae* Singer ex Singer, and *Vaginatae* (Fr.) Quél., subgenus *Amanitina* (E.J. Gilbert) E.J. Gilbert, containing sections *Amidella* (J.E. Gilbert) Konrad et Maubl., *Arenariae* Zhu L. Yang, Y.Y. Cui et Q. Cai, *Phalloideae* (Fr.) Quél., *Roanokenses* Singer ex Singer, *Strobiliformes* Singer ex Q. Cai, Zhu L. Yang et Y.Y. Cui, and *Validae* (Fr.) Quél., and subgenus *Lepidella* Beauseigneur, containing section *Lepidella* Corner et Bas only.

Species in sect. *Vaginatae* are recognised by a combination of the following features: a striate and non-appendiculate pileus, a bulbless stipe base with saccate volva or warts arranged in incomplete belts, a mostly absent annulus, inamyloid basidiospores, and absent clamps (Yang 1997, 2005, Cui et al. 2023). There are a few African taxa in this section with an annulus, but they lack clamp connections (Tang et al. 2015).

Shorea robusta, commonly referred to as 'Sal', holds significant economic importance as a prevalent dipterocarp hardwood in India. It constitutes a substantial proportion of moist deciduous broadleaved tropical forests across the country. The species is primarily found in the plains, lower foothills, and valleys of the Himalayas, spanning regions in central, eastern, and north-western India, including Assam, West Bengal, Odisha, Madhya Pradesh, Chhattisgarh, Haryana, Himachal Pradesh, and Uttarakhand (Singh et Singh 1992, Kumar et Atri 2019). Macrofungal forays conducted in India have identified the association of Sal trees with species from diverse fungal genera such as *Amanita* Pers., *Boletellus* Murrill, *Borofutus* Hosen et Zhu L. Yang, *Craterellus* Pers., *Indoporus* A. Parihar, K. Das, Hembrom et Vizzini, *Lactarius* Pers., *Phylloporus* Quél., *Pisolithus* Alb. et Schwein., *Russula* Pers., and *Thaxterogaster* Singer (Ghosh et al. 2023a, 2023b, 2023c). In tropical Sal-dominated forest ecosystems in India, *Amanita* stands out as one of the prevalent ectomycorrhizal genera. However, the identification of these *Amanita* species has often relied on a broad similarity to their European and North American counterparts, lacking a thorough comparative assessment of detailed morphology and molecular data (Singha et al. 2017, Verma

et Pandro 2018, Verma et al. 2020). Unfortunately, in-depth taxonomic studies of these Sal-associated *Amanita* species have not yet been undertaken in India.

During macrofungal forays in rainy seasons, an interesting species of the genus *Amanita* was collected from different parts of Sal-dominated tropical forest areas of West Bengal and Jharkhand. Thorough morphological examinations and multi-gene (ITS, LSU, and *tef* 1-α) molecular phylogenetic analyses of these collections revealed an undescribed taxon of the genus *Amanita*. This novel taxon is proposed herein as *A. indovaginata*.

MATERIAL AND METHODS

Morphological studies. Fresh specimens were macromorphologically fully described, and images of the basidiomata were taken with a Nikon D5300 digital camera and a Canon Power Shot SX 50 HS camera. Colours were coded using the Methuen Handbook of Colour (Kornerup et Wanscher 1978). The collected samples were then dried in a wooden dryer at a temperature of about 50–55 °C. All microscopic features were observed from a freehand section of preserved dried material in a mixture of 1% ammoniacal Congo red and 1% Phloxine, after a short treatment in a warm aqueous 5% KOH solution to dissolve the gelatinous matrix and improve tissue dissociation. Melzer's reagent was used to check the amyloidity of basidiospores. Drawings of micromorphological elements were made with a Camera Lucida at 2000× magnification. Photomicrographs of the various elements were taken with a digital camera attached to an Olympus CH20i and an Olympus CX33 compound microscope (Olympus, Tokyo, Japan). Sixty basidiospores were measured from each of the three specimens in Melzer's reagent (Largent et al. 1977). Biometric variables followed Yang (1997) and Cui et al. (2018). In the description of the basidiospores, the indication [n/m/p] represents n basidiospores measured from m basidiomata of p collections. Dimensions for basidiospores are given using a range notation in the form (a)b–c(d) where 'b–c' contains a minimum of 90% of the measured values (5th to 95th percentile). Extreme values, a or d, are given in parentheses. Q represents the length/width ratio of a basidiospore in side view. Qm means the average Q of all basidiospores. Specimens were deposited at the Central National Herbarium (CAL), Howrah.

DNA extraction, polymerase chain reaction (PCR) and sequencing. Genomic DNA was extracted from 100 mg of a dried basidiome with the InstaGene™ Matrix Genomic DNA isolation kit (Biorad, Hercules, USA) following the manufacturer's instructions. PCR amplification of the ITS region, part of the LSU, and *tef* 1-α were performed using primer pairs ITS1-F and ITS4 (White et al. 1990), LR0R and LR5 (Gardes et Bruns 1993), and ef1-983F and ef1-1567R (Rehner et Buckley 2005), respectively. PCR amplification was carried out in a ProFlex PCR system (Applied Biosystems, Foster City, USA) programmed for an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min. The final extension was kept at 72 °C for 7 min. The PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Both strands of the PCR fragment were sequenced on an ABI 3500 DNA Analyzer (Applied Biosystems, Foster City, USA) using the amplifying primers. The sequence quality was checked using Sequence Scanner Software v. 1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v. 5.1 (Drummond et al. 2010). In this study, six sequences (two each for ITS, LSU, and *tef* 1-α) were generated from two separate collections (CAL 1921 and CAL 1964) and deposited in GenBank (Tab. 1).

Tab. 1. List of species, specimen voucher and sequence accession numbers of species used in this study. The sequences were retrieved from GenBank, except for two UDB sequences retrieved from UNITE.

Species name	Specimen voucher	GenBank/UNITE accession nos.			Reference
		ITS	LSU	*tef* 1-α	
Amanita caesarea	RET 450-3	—	KF877208	—	Sánchez-Ramírez et al. 2015
Amanita caesareoides	S-292	LC056757	LC056757	—	Endo et al. 2016
Amanita cattaraugana	LVK14033	KX261519	KX261520	—	Unpublished
Amanita chiui	HKAS76328	MH508303	MH486447	MH508727	Cui et al. 2018
Amanita circulata	HKAS97543	OR042765	OR042725	OR046361	Cui et al. 2023
Amanita circulata	HKAS127629	OR042763	OR042717	OR046356	Cui et al. 2023
Amanita constricta	BW Mycoblitz IV 2	—	HQ539684	—	Unpublished
Amanita cornelii	CAL 1337	—	NG_066371	—	Tibpromma et al. 2017
Amanita daimonioctantes	RET 384-3	MK522023	KP284297	—	Tulloss et Yang 2018
Amanita dhakuriana	TM_1359	—	MH107236	—	Mehmood et al. 2018
Amanita emodotrygon	CAL 1338	—	NG_148940	—	Tibpromma et al. 2017
Amanita flavidocerea	BZ2015-59	—	MF461559	—	Thongbai et al. 2018
Amanita flavidocerea	BZ2015-60	—	MF461560	—	Thongbai et al. 2018
Amanita fuligineodisca	ANDES_F404 AFM1812	FJ890027	FJ890039	—	Vargas et al. 2011
Amanita fulva	KA12-1406	KF017933	KF021672	—	Kim et al. 2013
Amanita glarea	SJ44	KY781174	KY781175	—	Jabeen et al. 2017
Amanita glarea	SUA711 (LAH35079)	KY817313	KY817314	—	Jabeen et al. 2017
Amanita griseofolia	HKAS:38159	NR_119498	NG_042401	—	Zhang et al. 2004
Amanita griseofusca	AKA12	—	OL702871	—	Kumar et al. 2021
Amanita griseofusca	LAH35366	MH241055	MH241056	—	Kiran et al. 2018
Amanita indovaginata	**CAL 1921**	**OQ852757**	**OQ847820**	**OR801232**	**This study**
Amanita indovaginata	**CAL 1964**	**OQ852761**	**OQ847821**	**OR801233**	**This study**
Amanita jakeslandingensis	RET 397-5	KX018798	KX018804	—	Tulloss et Yang 2018
Amanita justicei	RET 697-5	KY614230	KY614226	—	Tulloss et Yang 2018
Amanita justicei	RET 691-2	KY614229	KY614225	—	Tulloss et Yang 2018
Amanita kryorhodon	RET 691-9	KY435397	KY435398	—	Tulloss et Yang 2018
Amanita liquii	HKAS:54568	KU714559	JF710794	KU714525	Zhang et al. 2004
Amanita liquii	HKAS93915	MH508427	MH486628	MH508886	Zhang et al. 2004
Amanita lividopallescens	RET 306-2	MW013159	MW013165	—	Tulloss et Yang 2018
Amanita luzernensis	RET 704-6	KY799116	KY799115	—	Tulloss et Yang 2018
Amanita madagascarensis	PC 0084419	—	JQ512086	—	Tang et al. 2015
Amanita madagascarensis	PC 0084421	—	JQ512087	—	Tang et al. 2015
Amanita mortenii	RET 294-8	KT317713	KT317710	—	Tulloss et Yang 2018
Amanita multicingulata	HKAS127630	OR042750	OR042713	OR046352	Cui et al. 2023

Species name	Specimen voucher	GenBank/UNITE accession nos.			Reference
		ITS	LSU	*tef* 1-α	
Amanita multicingulata	HKAS128049	OR042751	OR042712	OR046351	Cui et al. 2023
Amanita myrmeciae	RET 495-10	—	KU186806	—	Tulloss et Yang 2018
Amanita myrmeciae	RET 473-8	—	MF422623 & MN518731	—	Tulloss et Yang 2018
Amanita myrmeciae	RET 687-7	—	KU852505	—	Tulloss et Yang 2018
Amanita olivaceogrisea	RET 523-2	KU867878	KU867877	—	Tulloss et Yang 2018
Amanita orientalis	EFHAAU1367	OR042759	OR042698	—	Cui et al. 2023
Amanita orientifulva	KA12-0642	KF017940	KF021679	—	Kim et al. 2013
Amanita penetratrix	RET 704-1	KU186828	KU186832	—	Tulloss et Yang 2018
Amanita penetratrix	RET 704-8	KU186830	KU186834	—	Tulloss et Yang 2018
Amanita populiphila	RET 103-6	KP224321	KP224344	—	Tulloss et Yang 2018
Amanita populiphila	RET 035-8	KP221305	KP221314	—	Tulloss et Yang 2018
Amanita pseudovaginata	HKAS70138	MH508531	MH486791	MH509017	Cui et al. 2018
Amanita rajendrae	CAL 1530	—	NG_057144	—	Das et al. 2017
Amanita rhacopus	RET 421-1	KU186824	KU186820	—	Tulloss et Yang 2018
Amanita semiobruta	RET 691-1	KY435402	KY435401	—	Tulloss et Yang 2018
Amanita shennongjiana	HKAS75553	MH508590	MH486862	MH509085	Cui et al. 2018
Amanita sinicoflava	BW PH061306-1	—	HQ539739	—	Tulloss et Yang 2023
Amanita sinofulva	HKAS92355	OR042741	OR042703	OR046345	Cui et al. 2023
Amanita sp.	RET 690-10	KY614233	KY614227	—	Tulloss et Yang 2018
Amanita sp.	RET 716-3	KY952149	KY952148	—	Tulloss et Yang 2018
Amanita sp.	Kew 29799	JF710841	JF710817	—	Tang et al. 2015
Amanita sp.	RET 409-9	—	KX443409	—	Tulloss et Yang 2018
Amanita sp.	RET 345-3	KU186807	KU186808	—	Tulloss et Yang 2018
Amanita sp.	RET 692-2	KY349229	KY349232	—	Tulloss et Yang 2018
Amanita sp.	SH0944958. 09FU*	UDB01056109	—	—	Unpublished
Amanita sp.	SH0031588. 09FU*	UDB01056112	—	—	Unpublished
Amanita strobilaceovolvata	PC 0084418	—	JQ512085	—	Tang et al. 2015
Amanita subtropicana	TM 15-995	—	MG923799	—	Phookamsak et al. 2019
Amanita subtropicana	TM 17-1574	—	MG913204	—	Phookamsak et al. 2019
Amanita supravolvata	RET 103-7	NR_154693	—	—	Tulloss et Yang 2018
Amanita texasorora	RET 581-6	KP662531	KP662533	—	Tulloss et Yang 2018
Amanita trygonion	RET 622-5	KU186809	KU186810	—	Tulloss et Yang 2018
Amanita vaginata	KA12-0962	KF017950	KF021689	—	Kim et al. 2013
Amanita williamsiae	RET 160-3	—	KX270330	—	Tulloss et Yang 2018
Amanita williamsiae	RET 478-10	—	KX270332	—	Tulloss et Yang 2018
Amanita xanthomitra	RET 645-4	KX270323	KX270342	—	Tulloss et Yang 2018
Amanita xanthomitra	RET 652-7	KX270325	KX270343	—	Tulloss et Yang 2018

* species hypotheses from environmental samples, not documented by a voucher

Sequence alignment and phylogenetic analysis. The ITS, LSU, and *tef* 1-α sequences of the newly described *Amanita* species plus close relatives were retrieved from an nBLAST search against GenBank (https://www.ncbi.nlm.nih.gov/genbank), UNITE database (https://unite.ut.ee), and relevant published phylogenies (Tab. 1). Three raw datasets (ITS, LSU, and *tef* 1-α) were created separately. All the datasets were aligned separately using the online version of the multiple sequence alignment program MAFFT v. 7 (https://mafft.cbrc.jp/alignment/software/) with the L-INS-i strategy and normal alignment mode, respectively. The alignment was checked and trimmed with the conserved motifs manually with MEGA v. 7 (Kumar et al. 2016). To eliminate poorly aligned regions in the ITS alignment, TrimAl (Capella-Gutiérrez et al. 2009) was used. Furthermore, three alignments (ITS, LSU, and *tef* 1-α) were concatenated into a multi-locus dataset using BioEdit v. 7.0.9 (Hall 1999) and processed for the phylogenetic analyses. In the three-locus dataset (ITS + LSU + *tef* 1-α) of *Amanita*, sequence lengths were determined to be 421 bp for ITS, 948 bp for LSU and 487 bp for *tef* 1-α. In our present phylogenetic analysis, the three-locus dataset consisted of 71 taxa and 1,856 nucleotide sites, including gaps, with *Amanita caesarea* (Scop.) Pers. and *A. caesareoides* Lj.N. Vassiljeva as outgroup taxa. The combined dataset contained 848 distinct patterns, 559 parsimony-informative, 157 singleton sites, and 1140 constant sites. The combined dataset was phylogenetically analysed using both the Maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood analysis was conducted using the IQ-tree tool v. 2.2.2.6 (Nguyen et al. 2015), employing the best model for each locus chosen by ModelFinder (Kalyaanamoorthy et al. 2017). Additionally, ultrafast bootstrap with 10,000 replicates was applied to obtain nodal support values. Bayesian inference was computed in MrBayes v. 3.2.6 (Ronquist et al. 2012) with four Markov chain Monte Carlo (MCMC) algorithms. PartitionFinder2 was used to find the best nucleotide substitution models using the Bayesian information criterion (BIC) with a greedy search over all models (Lanfear et al. 2017). Two MCMC runs of four chains were executed simultaneously from a random starting tree for 2,000,000 generations until the standard deviation of split frequencies dropped below the 0.01 threshold. Trees were sampled every 100th generation. The first 25% of trees were discarded as burn-in. Chain convergence was determined using Tracer 1.5 (Rambaut et al. 2014) to ensure sufficiently large effective sample size (ESS) values (>200). Gaps in the alignment were treated as missing data in phylogenetic analyses. Phylogenetic analyses (ML and BI) were carried out with three loci (ITS + LSU + *tef* 1-α) and two loci (ITS + LSU). However, both ML and BI analyses resulted in essentially the same tree topologies, so we opted to include the three-gene phylogenetic analysis in the present communication instead of the two-gene analysis.

RESULTS

PHYLOGENETIC INFERENCES

Three-locus phylogenetic analysis revealed that the two collections of our species, *A. indovaginata* (voucher nos. CAL 1921 and CAL 1964), are clustered with *A. myrmeciae* Tulloss, Kudzma et Albertella originating from Australia (voucher nos. RET 687-7, RET 473-8 and RET 495-10) with strong support (MLbs = 89%), being sister to the significantly supported clade (MLbs = 100%, BPP = 1) containing two unnamed *Amanita* species (accession nos. UDB01056109 and UDB01056112) from India. Furthermore, this forms another sister clade with several other taxa, viz *A. circulata* Y.Y. Cui, Q. Cai et Zhu L. Yang (voucher nos. HKAS97543 and HKAS127629) and *Amanita justicei* Tulloss et Kudzma (voucher nos. RET 691-2 and RET 697-5) with strong (MLbs = 99%, BPP = 1) support (Fig. 1).

Fig. 1. Phylogram generated by Maximum likelihood analysis based on combined sequence data of ITS, LSU, and *tef* 1-α for *Amanita indovaginata* and allied species of *Amanita* sect. *Vaginatae*, with species of sect. *Caesareae* as outgroup. The novel taxon is highlighted using a bold red font. Maximum likelihood bootstrap (MLbs) values ≥ 70% and Bayesian posterior probability (BPP) values ≥ 0.95 are shown in the phylogenetic tree.

TAXONOMY

Amanita indovaginata A. Ghosh, A. Kumar, D. Chakr. et Hembrom, **sp. nov.**
Figs 2, 3

MycoBank: MB 848554

Etymology. The species epithet '*indo*' refers to the locality of the type specimen from India and '*vaginata*' refers to its similarity with *Amanita vaginata*.

Diagnosis. *Amanita indovaginata* differs from all known species of *Amanita* sect. *Vaginatae* by a combination of the following characters: brownish grey pileus with faded chalky white margins, subglobose to broadly ellipsoid basidiospores (11–14 × 10–12 µm), occurrence under *Shorea robusta* in tropical dry deciduous forest, and differences in ITS, LSU, and *tef* 1-α sequence data.

Holotype. India, Jharkhand, Sahibganj District, Durgapur Panchayat, Ulghutu area, village of Partee Pahar, 24°48'06" N, 87°43'05" E, elev. 62 m, on soil, under *Shorea robusta* in tropical dry deciduous forest, 18 September 2022, coll. A. Ghosh, AGJH-036 (CAL 1921).

Gene sequences: OQ852757 (ITS, from holotype), OQ852761 (ITS); OQ847820 (LSU, from holotype), OQ847821 (LSU); OR801232 (*tef* 1-α, from holotype), OR801233 (*tef* 1-α).

Description. Pileus 30–60 mm in diam., initially hemispherical, becoming convex to planoconvex with age, depressed at centre; surface dry, viscid when moist, smooth, brownish grey (7–9C–D2) at centre, gradually faded chalky white (1A1) towards margin, covered with greyish brown (7E3–4) minute fibrils, mostly lacking universal veil remnants; margin striate for 15–18 mm (up to 1/3 radius), incurved. Pileus context chalky white (1A1), up to 2 mm thick, thinning towards margin, unchanged when bruised or exposed. Lamellae free, crowded (8–14/cm at the pileus margin), chalky white (1A1), forked near the margin; lamellar edges concolorous with lamella surface. Lamellulae truncate, plentiful. Stipe 45–120 × 6–12 mm, cylindrical to subclavate, faded chalky white (1A1) to brownish grey (7–9C–D2), covered with white minute fibrils; context chalky white (1A1), hollow in the centre; basal bulb absent. Volva saccate, 16–50 × 10–27 mm, membranous, outer and inner surfaces chalky white to greyish white (1A1–B1). Annulus absent. Smell and taste not recorded. Spore print white (1A1).

Basidiospores [60/3/2] (10.5)11–14(15) × (9.5)10–12(13) µm [Q = (1.00)1.10–1.25(1.35); Qm = 1.16], subglobose to broadly ellipsoid, non-amyloid, hyaline, thin-walled, smooth, apiculus sublateral, up to 1 µm high; contents monoguttulate. Basidia 46–73 × 14–18 µm, 2- and 4-spored, thin-walled, colourless; sterigmata 3–7 µm long; clamp connections at the base of basidia not observed. Lamellar edge sterile, with inflated thin-walled, hyaline, subglobose to pyriform cells (25–45 × 12–40 µm). Subhymenium 20–55 µm thick; basidia arising from subglobose to irregularly shaped cells. Hymenophoral trama bilateral, 30–50 µm wide, composed of inflated clavate cells (20–40 × 8–12 µm); filamentous, undifferentiated hyphae 3–7 µm wide, hyaline. Pileipellis 95–115 µm thick, in two layers; gelatinised suprapellis 40–57 µm thick, composed of irregularly arranged, gelatinised, colourless, hyaline, thin-walled, filamentous hyphae 4–6 µm wide; subpellis 55–70 µm thick,

Fig. 2. *Amanita indovaginata* (CAL 1921, holotype): **A–B** – young and mature basidiomata in situ, **C–D** – basidia and elements of subhymenium, **E–F** – basidiospores in Melzer's reagent and Congo red, **G–H** – longitudinal section of the outer part of volval remnants on the stipe base. Scale bars = 10 µm. Photos by Aniket Ghosh (A, B), Anil Kumar (C–H).

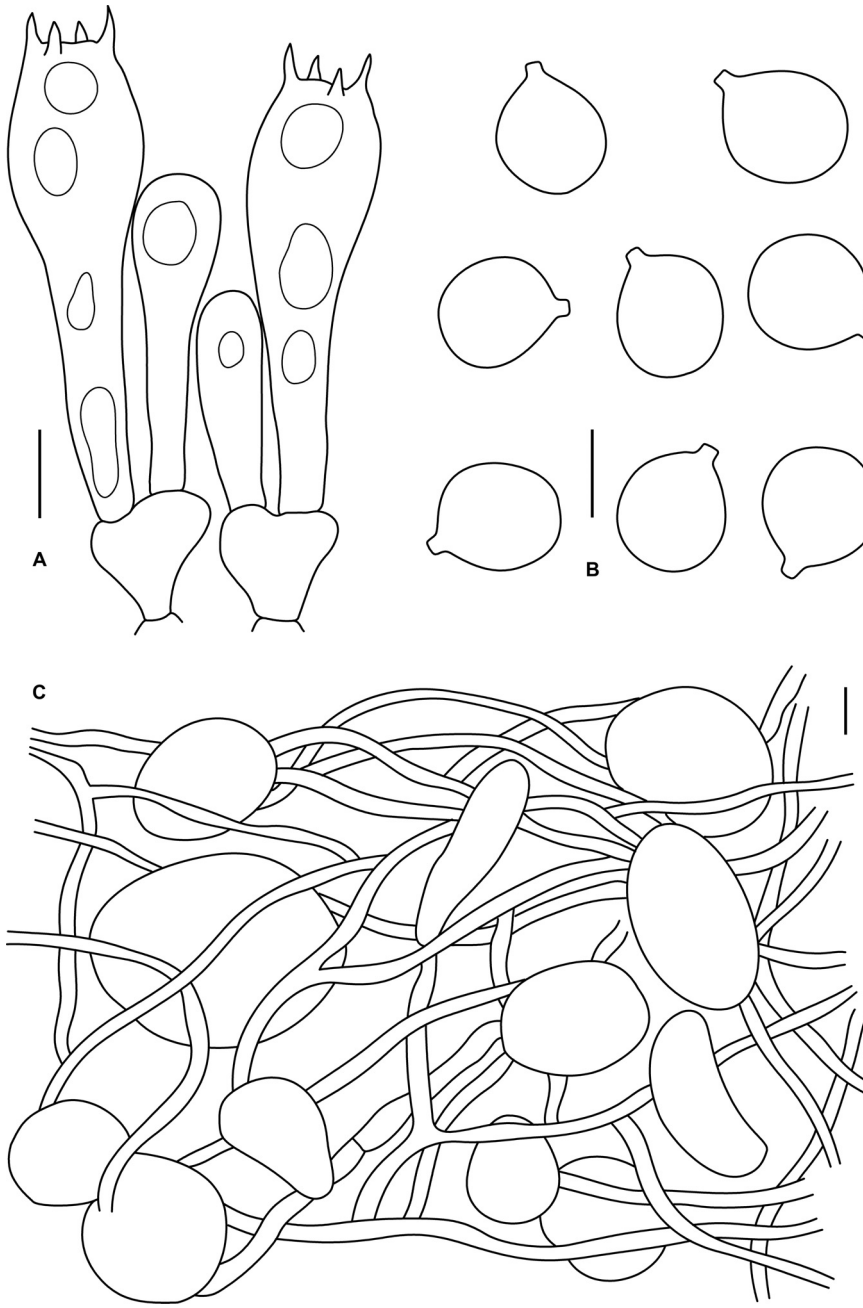

Fig. 3. Microscopic features of *Amanita indovaginata* (CAL 1921, holotype): **A** – basidia and elements of subhymenium, **B** – basidiospores, **C** – longitudinal section of the outer part of volval remnants on the stipe base. Scale bars = 10 µm. Drawings by Anil Kumar.

non-gelatinised, composed of filamentous hyphae 4–7 µm wide, radially arranged, with yellowish brown intracellular pigments. Pileus trama composed of undifferentiated filamentous hyphae (3–8 µm wide) with broadly ellipsoid to ellipsoid cells (20–60 µm wide). Outer surface of universal veil (on stipe base) composed of longitudinally arranged elements: filamentous hyphae predominant, 3–6 µm wide, rarely or occasionally septate, slightly thick-walled, branched, anastomosing; inflated cells globose to subglobose (50–65 × 40–48 µm) or ellipsoid (40–68 × 25–43 µm), colourless, thin-walled, hyaline. Inner surface of universal veil (on stipe base) composed of filamentous hyphae 3–5.5 µm wide; inflated cells subglobose to ovoid (55–80 × 45–50 µm), infrequent, no clamps observed. Stipe context longitudinally acrophysalidic; acrophysalides 102–204 × 20–28 µm; filamentous, undifferentiated thin-walled, hyaline hyphae 2–8 µm wide. Clamp connections absent in all tissues.

Habitat. Solitary to scattered, growing in association with *Shorea robusta* in tropical deciduous forests.

Additional specimen examined

India. West Bengal, Uttar Dinajpur District, town of Kaliyaganj, Dhamja forest, 25°18'00" N, 88°20'35" E, elev. 80 m, on soil, under *Shorea robusta* in tropical dry deciduous forest, 18 June 2022, coll. A. Ghosh et D. Chakraborty, RGJ-22-02 (CAL 1964).

Discussion

The combination of macro- and micromorphological features, such as the striate and non-appendiculate pileus, absence of an annulus, the bulbless stipe base with saccate volva, absence of clamps, and inamyloid basidiospores, place *A. indovaginata* in *Amanita* subg. *Amanita* sect. *Vaginatae* sensu Cui et al. (2023).

Amanita myrmeciae Tulloss, Kudzma et Albertella, *A. justicei* Tulloss et Kudzma, *A. circulata* Y.Y. Cui, Q. Cai et Zhu L. Yang, and *A. williamsiae* Tulloss, Kudzma et J.L. Horman are phylogenetically closely related to the new species (Fig. 1). However, all of these are distinguished morphologically. *Amanita myrmeciae*, originally described from the USA, has a light brownish to brown pileus, subglobose basidiospores (9–11.2 × 8–10 µm in size) and associates with species of *Eucalyptus* (Tulloss et Yang 2023). *Amanita justicei*, also originally described from the USA, has a yellow to yellow-brown pileus with a distinct umbo and is associated with *Pinus* and *Quercus* stands (Tulloss et Yang 2023). On the other hand, *A. circulata*, originally described from China, can easily be distinguished from the present taxon by its grey brown to dark brown pileus with distinctly brownish ring-like zone at the proximal end of marginal striations (Cui et al. 2023). *Amanita williamsiae* can be differentiated by having a yellow pileus

with a prominent tannish umbo and its occurrence in the *Pinus* and *Quercus* stands (Tulloss et Yang 2023).

Morphologically, *Amanita indovaginata* is somewhat similar to *A. vaginata* (Bull.) Lam. However, the latter can be easily distinguished from the former species by its pileus colour, which is bistre-grey to brownish grey (Kibby 2003), unlike *A. indovaginata*. On the other hand, *Amanita glarea* Jabeen, Kiran et Sadiqullah, originally reported from Pakistan, might be confused with the present taxon due to its greyish brown pileus, off-white towards the margins, but differs from the present taxon by its globose to subglobose basidiospores [(10)10.1–11.7(12) × (9.6)10.4–11(11.5) µm], with lower Q value = 1.08 and its association with species of *Cedrus*, *Pinus*, and *Abies* in coniferous forests (Jabeen et al. 2017).

Amanita indovaginata shares similarities with several Indian species in the sect. *Vaginatae*, including *A. cornelii* Olariaga, D. Rodr., G. Moreno, Manjón, Salcedo, V. Hofst. et Buyck, *A. dhakuriana* Mehmood, K. Das, Hosen, R.P. Bhatt et U. Singh, *A. emodotrygon* Mehmood, Tulloss, K. Das, Hosen et R.P. Bhatt, *Amanita rajendrae* Mehmood, K. Das, Uniyal, and *A. subtropicana* Mehmood et R.P. Bhatt. However, *A. cornelii* is easily distinguished by its pileus with an irregular pattern of radially oriented brown stripes, its stipe covered with grey to brown fibrils, and the globose to subglobose basidiospores (9–11.5 × 8–10.5 µm) with a lower Q value = 1.06 (Tibpromma et al. 2017). *Amanita dhakuriana* has a greenish grey to yellowish grey umbonate pileus and its association with species of *Abies* and *Quercus* in mixed forests (Mehmood et al. 2018). *Amanita emodotrygon* has a greyish brown to olive, campanulate pileus with a deeply grooved margin, crowded white to yellowish lamellae and is typically found under *Pinus roxburghii* in coniferous forests (Tibpromma et al. 2017). *Amanita rajendrae* is distinguished by its pale yellow to light yellow pileus, stipe covered with fibrils arranged in a zigzag pattern, globose basidiospores (8.7–10.5 × 8–10 µm) and its occurrence under *Cedrus deodara*. *Amanita subtropicana*, reported from the same host (i.e. *Shorea robusta*), is easily distinguished from *A. indovaginata* by its olive-brown to yellowish brown pileus covered with a felty, subconical, subpyramidal to warty universal veil remnants, and its broadly ellipsoid to ellipsoid basidiospores [(7)8.5–11(12) × (5.5)6–8(8.5) µm] with a higher Q value = 1.41 (Phookamsak et al. 2019).

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