Three new species of genus Amanita Pers. (Amanitaceae) from India

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Research Article

Keywords: new taxa, morphology, molecular phylogeny, taxonomy

Posted Date: May 8th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2873043/v1

Abstract

Based on morphological evidence and DNA sequencing, we propose three new species of the genus *Amanita*, namely *Amanita aurantialba, A. indovaginata* and *A. pseudohemibapha* from India. *Amanita aurantialba*, a member of sect. *Phalloideae*, *A. indovaginata* a representative of sect. *Vaginatae* and *A. pseudohemibapha*belong to sect. *Caesareae*. *Amanita aurantialba* was collected from the temperate forests dominated by *Quercus* species, whereas *A. pseudohemibapha* and *A. indovaginata* are associated with tropical forests dominated by *Shorea robusta*. Photographs, line drawings and detailed morphological descriptions for these species are provided with comparisons against closely-related taxa.

Introduction

The genus *Amanita* is one of the most speciose genera in the *Amanitaceae* family, distributed in all continents except Antarctica and exhibits a worldwide distribution from the tropics to the Nearctic (Codjia et al. 2023). To date, 1000 *Amanita* species names have been proposed to date, but 690 species are accepted worldwide (Yang et al. 2018; Cui et al. 2018; Kalichman et al. 2020). There are approximately 66 species of *Amanita* reported in India (Mehmood et al. 2018; Hosen et al. 2018; Kumar et al. 2022, 2023). The temperate coniferous and subtropical broad-leaved forests of India harbour high mushroom diversity including genus *Amanita*.

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

Species in sect. *Vaginatae* are recognized by the combination of the following features: the sulcate margin of the pileus, a stipe with neither an annulus nor a bulbous base, and the absence of clamp connections at the base of basidia (Yang 1997; 2005). There are a few African taxa in this sect. with an annulus, but without clamp connections (Tang et al. 2015). According to Bas (1969), species within sect. *Phalloideae* are usually recognized by the combination of the following features: white-colored basidiomata, non-striated, appendiculate pileus margin a limbate or saccate volva on the bulbous stipe base (Bas 1969). Members of sect. *Caseareae* are identified by the following characteristics: striate pileus margins, truncate lamellulae, saccate volva, presence of an annulus, inamyloid basidiospores, and abundant connections at the basidia bases (Yang 1997).

During the rainy season, three interesting species of the genus *Amanita* were collected from mixed coniferous and deciduous forests dominated by *Quercus* sp. and *Shorea robusta* in India. Morphological

and molecular analyses revealed that the collections described here represent three new species to science.

Materials and Methods

The studied survey sites were located in the northern and eastern Indian subcontinents (Indian states of Jammu and Kashmir, Jharkhand and West Bengal). Fruiting bodies were collected yearly from 2021 onwards between July and September. Geographic coordinates were recorded using a Garmin e-Trax30 hand-held GPS receiver.

Macro and micromorphology

Fresh specimens were macromorphologically fully described, and images of the basidiomata were taken with a Nikon D5300 digital camera and Canon Power Shot SX 50 HS cameras. Colours were coded using the Methuen Handbook of Colour (Kornerup & 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 free hand section of preserved dried tissues 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. Drawings of micromorphological elements were made with a Camera Lucida at 2000× magnification. Photomicrographs of the various elements were captured with a digital camera attached to an Olympus CH20i and an Olympus CX33 compound microscope. 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). The ranges of the measurements of the basidiospores are presented as '(a) b–c (d)', where the range 'b–c' has a minimum of 90% of the measurements, while 'a' and 'd' in brackets are extreme values. 'Q' stands for the ratio of length and width of basidiospores in side view, and 'Qm' means the average 'Q' of all basidiospores.

DNA extraction, PCR amplification and sequencing

A Plant II Kit (Macherey-Nagel) was used to isolate nuclear genomic DNA from 100 mg of dried basidiomata. The universal primer pairs ITS1-F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC – 3') were used to amplify the ITS region of the nuclear ribosomal DNA (White et al. 1990, Gardes & Bruns 1993). For amplification of the LSU region, the primer pair LR0R (5'-ACCCGCTGAACTTAAGC – 3') and LR5 (5'-TCCTGAGGGAAACTTCG-3') were used (Vilgalys & Hester 1990). PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X Phire PCR buffer (contains 1.5 mM MgCl2), 0.2 mM each dNTPs, 1 µl DNA, 0.2 µl Phire Hotstart II DNA polymerase enzyme, 0.1 mg/ml BSA, 3% DMSO, 0.5M Betaine, and 5pM of forward and reverse primers. Thermal profile of PCR for ITS and large subunit (LSU) was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) programmed for 2 min at 96°C, followed by 30 cycles of 30 sec at 96°C, 40 sec at 50°C, 2 min at 72°C, and a final 7 min extension step at 72°C. All newly generated ITS and LSU sequences were deposited in GenBank and accession numbers procured. Accession numbers of species used in the phylogenetic analysis are listed in the Table 1.

Table 1
Collection information of voucher specimen and GenBank accession numbers for sequences used in phylogenetic analyses

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita albolimbata	JEIC0653	Benin	MT966933	MT966940
Amanita albolimbata	JEIC0667	Benin	MT966932	MT966939
Amanita albolimbata	JEIC0638	Benin	MT966931	MT966938
Amanita albolimbata	JEIC0707	Benin	MT966936	MT966943
Amanita albolimbata	EIC0739	Benin	MT966935	MT966942
Amanita albolimbata	EIC0675	Benin	MT966934	MT966941
Amanita albolimbata	HKAS94241	Benin	—	MT966944
Amanita harkoneniana	P Pirot SN	Madagascar	MK570922	MK570929
Amanita harkoneniana	TS 1061	Tanzania	MK570923	MK570930
Amanita alliodora	DNS062	Madagascar	KX185611	KX185612
Amanita bweyeyensis	TS591	Tanzania	MK570921	MK570928
Amanita bweyeyensis	JD1304	Rwanda	MK570920	MK570927
Amanita bweyeyensis	JD1257	Rwanda	MK570919	MK570926
Amanita fuligineoides	HKAS52727	China	JX998024	JX998047
Amanita fuligineoides	HKAS83694	China	—	MH486553
Amanita parviexitialis	HKAS79049	China	KJ466389	KJ466449
Amanita exitialis	HKAS74673	China	KJ466375	KJ466435
Amanita exitialis	HKAS75776	China	JX998025	JX998051
Amanita exitialis	HKAS75774	China	JX998027	JX998052
Amanita exitialis	HKAS75775	China	JX998026	JX998053
Amanita suballiacea	RET490-1	USA	KJ466420	KJ466485
Amanita suballiacea	RET478-6	USA	KJ466419	KJ466484
Amanita suballiacea	RET491-7	USA	KJ466421	KJ466486
Amanita rimosa	HKAS77279	China	KJ466392	KJ466454
Amanita rimosa	HKAS77335	China	KJ466393	KJ466455

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita rimosa	HKAS77120	China	MH508547	KJ466453
Amanita rimosa	HKAS101393	China	——	MH486806
Amanita bisporigera	RET377-9	USA	KJ466374	KJ466434
Amanita pallidorosea	HKAS77349	China	KJ466389	KJ466449
Amanita pallidorosea	HKAS82350	China	MH508485	MH486737
Amanita pallidorosea	HKAS75483	China	KJ466384	KJ466445
Amanita pallidorosea	HKAS75786	China	JX998037	JX998054
Amanita aurantialba	**AKA31**	**India**	**OQ875738**	**OQ553808**
Amanita aurantialba	**AKS23**	**India**	——	**OQ561447**
Amanita fuliginea	HKAS75780	China	JX998023	JX998048
Amanita fuliginea	HKAS75782	China	JX998022	JX998049
Amanita fuliginea	HKAS75781	China	JX998021	JX998050
Amanita fuliginea	HKAS79685	China	KJ466377	KJ466437
Amanita subjunquillea	HKAS75771	China	JX998032	JX998063
Amanita subjunquillea	HKAS75770	China	JX998034	JX998062
Amanita subjunquillea	HKAS75772	China	JX998033	JX998061
Amanita subjunquillea	HKAS77325	China	KJ466425	KJ466490
Amanita phalloides	HKAS75773	USA	JX998031	JX998060
Amanita phalloides	MB-102659	Germany	——	MH486754
Amanita ocreata	HKAS79686	USA	KJ466381	MH486688
Amanita virosa	HKAS71040	Japan	KJ466429	KJ466496
Amanita virosa	HKAS90176	China	MH508650	MH486948
Amanita subpallidorosea	LHJ140923-55	China	KP691680	KP691693
Amanita subpallidorosea	LHJ140923-41	China	KP691683	KP691692
Amanita subpallidorosea	LHJ140926-11	China	KP691682	KP691688
Amanita griseorosea	HKAS89004	China	——	KU168387
Amanita griseorosea	HKAS77332	China	KJ466411	KJ466474

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita griseorosea	HKAS77333	China	KJ466412	KJ466475
Amanita molliuscula	HMJAU20469	China	KJ466410	KJ466473
Amanita molliuscula	HKAS77324	China	KJ466409	KJ466472
Amanita molliuscula	HKAS75555	China	KJ466408	KJ466471
Amanita brunneitoxicaria	BZ2015 01	Thailand	KY747462	——
Amanita brunneitoxicaria	BZ2015 02	Thailand	KY747463	——
Amanita zangii	HKAS99663	China	MH508655	MH486958
Amanita zangii	GDGM29241	China	KJ466432	KJ466499
Amanita virginiana	RET-374-8	USA	JX844750	JX844750
Amanita fense	MHKMULP Tang1565	China	MT108788	MT138687
Amanita fense	MHKMULP Tang2269	China	MT108789	MT138688
Amanita ristichii	RET-096-1	Canada	JX844738	KF877276
Amanita longistriata	MHHNU 6993	China	KU714580	KU714542
Amanita incarnatifolia	HKAS100593	China	MH508400	MH486594
Amanita roseolamellata	RET 474-6	Australia	KP866165	KT006768
Amanita spreta	RET 234-6	USA	KX365200	KX365201
Amanita yuaniana	HKAS58807	China	MH508653	MH486954
Amanita hunanensis	MHHNU 30281	China	KU714581	KP276140
Amanita esculenta	HMLD 0339	China	KU714582	KP276141
Amanita torrendii	LOU-Fungi 17408	Spain	GQ925386	GQ925369
Amanita torrendii	HKAS 59739	Spain	KU714578	KU714555
Amanita pseudohemibapha	**AGJH-062**	**India**	**OQ847828**	**OQ847831**
Amanita pseudohemibapha	**AGJH-078**	**India**	**OQ847829**	**OQ847830**
Amanita hemibapha	TBGT Pradeep 5872	India	KU714567	JF710799
Amanita hemibapha	RET-342-8	India	JX844716	KF877233
Amanita hemibapha	RMUKK02	Thailand	MW403849	——
Amanita hemibapha	RMUKH01	Thailand	MW403849	——

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita hemibapha	KY288023	India	KY288023	------
Amanita hemibapha	RET 718-2	India	KY288023	------
Amanita hemibapha	TM 15–843	India	KY214405	KY214404
Amanita kitamagotake	EN-4	Japan	AB721450	------
Amanita similis	FRIM 69312	Malaysia	KU714573	JF710797
Amanita similis	FRIM 3740	Malaysia	KU714566	JF710796
Amanita subhemibapha	HKAS97518	China	MH508621	MH486907
Amanita fuscoflava	HKAS59800	China	MH508372	MH486557
Amanita rubroflava	HKAS83089	China	MH508568	MH486827
Amanita ochracea	HKAS87986	China	MH508454	MH486686
Amanita jacksonii	RET-393-6	USA	JX844725	KF877251
Amanita caesarea	RET 036 – 2	Italy	------	KF877205
Amanita caesarea	HKAS96166	Italy	MH508283	MH486418
Amanita caesareoides	HKAS92017	China	MH508286	MH486422
Amanita caesareoides	HKAS92009	China	MH508285	MH486421
Amanita malayensis	FRIM 61278	Malaysia	KU714574	KU714550
Amanita javanica	FRIM 61503	Malaysia	KU714572	KU714548
Amanita rubromarginata	HKAS 59740	China	KU714571	KU714547
Amanita masasiensis	H 7002979	Tanzania	JF710836	JF710805
Amanita loosii	PC 0084409	Burundi	JQ512092	------
Amanita chepangiana	HKAS 56718	China	KU714569	KU714545
Amanita pseudoprinceps	HKAS97520	China	MH508526	MH486787
Amanita aporema	FRIM 62674	Malaysia	KU714575	KU714551
Amanita princeps	FRIM 62849	Malaysia	KU714576	KU714552
Amanita calyptratoides	RET-273-7	USA	JX844691	KF877211
Amanita squarrosipes	HKAS76359	China	MH508613	MH486894.
Amanita imazekii	HKAS71045	Japan	MH508397	MH486591

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita murrilliana	RET-278-1	USA	JX844731	KF877269
Amanita ovalispora	HKAS79625	China	MH508479	MH486722
Amanita tenuifulva	HKAS58877	China	MH508635	MH486928
Amanita cingulata	HKAS100640	China	MH508309	MH486454
Amanita vaginata	KA12-0962	South Korea	KF017950	KF021689
Amanita cattaraugana	LVK14033	USA	KX261519	KX261520
Amanita sp	RET 690 – 10	USA	KY614233	KY614227
Amanita sp	RET 716-3	USA	KY952149	KY952148
Amanita cornelii	CAL 1337	India	----	NG_066371
Amanita rajendrae	CAL 1530	India	----	NG_057144
Amanita shennongjiana	HKAS75553	China	MH508590	MH486862
Amanita luzernensis	RET 704-6	USA	KY799116	KY799115
Amanita dhakuriana	TM_ 1359	India	----	MH107236
Amanita kryorhodon	RET 691-9	Canada	KY435397	KY435398
Amanita populiphila	RET 103-6	USA	KP224321	KP224344
Amanita populiphila	RET 035 – 8	USA	KP221305	KP221314
Amanita lividopallescens	RET 306-2	Germany	MW013159	MW013165
Amanita griseofusca	AKA12	India	-----	OL702871
Amanita griseofusca	LAH35366	Pakistan	MH241055	MH241056
Amanita chiui	HKAS76328	China	MH508303	MH486447
Amanita sp	Kew 29799	Malawi	JF710841	JF710817
Amanita sp	RET 409-9	USA	-----	KX443409
Amanita griseofolia	HKAS 38159	USA	NR_119498	NG_042401
Amanita fuligineodisca	AFM1812	Colombia	FJ890027	FJ890039
Amanita daimonioctantes	RET 384-3	USA	MK522023	KP284297
Amanita fulva	KA12-1406	South Korea	KF017933	KF021672
Amanita orientifulva	KA12-0642	South Korea	KF017940	KF021679

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita jakeslandingensis	RET 397-5	USA	KX018798	KX018804
Amanita xanthomitra	RET 645-4	USA	KX270323	KX270342
Amanita xanthomitra	RET 652-7	USA	KX270325	KX270343
Amanita constricta	BW Mycoblitz IV 2	USA	HQ539788	HQ539684
Amanita sinicoflava	BW PH061306-1	USA	HQ539843	HQ539739
Amanita mortenii	RET 294-8	Greenland	KT317713	KT317710
Amanita olivaceogrisea	RET 523-2	Germany	KU867878	KU867877
Amanita texasorora	RET 581-6	USA	KP662531	KP662533
Amanita rhacopus	RET 421-1	USA	KU186824	KU186820
Amanita subtropicana	TM 15–995	India	----	MG923799
Amanita subtropicana	TM 17-1574	India	----	MG913204
Amanita sp	RET 345-3	Zambia	KU186807	KU186808
Amanita strobilaceovolvata	PC 0084418	Zambia	----	JQ512085
Amanita madagascarensis	PC 0084419	Madagascar	----	JQ512086
Amanita madagascarensis	PC 0084421	Madagascar		JQ51208
Amanita sp	RET 692-2	Australia	KY349229	KY349232
Amanita myrmeciae	RET 495 – 10	Australia	----	KU186806
Amanita myrmeciae	RET 473-8	Australia	MF422623.	MN518731
Amanita myrmeciae	RET 687-7	Australia	----	KU852505
Amanita indovaginata	**AGJH-036**	**India**	**OQ852757**	**OQ847820**
Amanita indovaginata	**RGJ-22-02**	**India**	**OQ852761**	**OQ847821**
Amanita justicei	RET 697-5	USA	KY614230.	KY614226
Amanita justicei	RET 691-2	USA	KY614229	KY614225
Amanita williamsiae	RET 160-3	USA	----	KX270330
Amanita williamsiae	RET 478 – 10	USA	----	KX270332
Amanita semiobruta	RET 691-1	USA	KY435402	KY435401
Amanita penetratrix	RET 704-1	USA	KU186828	KU186832

Species name	Specimen voucher	Location	GenBank	
			nrITS	nrLSU
Amanita penetratrix	RET 704-8	USA	KU186830	KU186834
Amanita emodotrygon	CAL 1338	India	----	NG_148940
Amanita trygonion	RET 622-5	India	KU186809	KU186810
Amanita caesarea	RET 450-3	Italy	----	KF877208
Amanita caesareoides	S-292	Japan	LC056757	----

Phylogenetic analyses

The nrITS and nrLSU sequences of the newly generated three *Amanita* species (*Amanita aurantialba*, *A. indovaginatae* and *A. pseudohemibapha*) and their close relatives were retrieved from nBLAST search against GenBank (https://www.ncbi.nlm.nih.gov/genbank) (https://unite.ut.ee) and relevant published phylogenies (Tibpromma et al. 2017). Two datasets (nrITS and nrLSU) 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 (Katoh et al. 2019). The alignment was checked and trimmed manually with MEGA v. 7 (Kumar et al. 2016). To eliminate ambiguous positions in the alignment as much objectively as possible, Gblocks 0.91b (Talavera & Castresana 2007) was used. The program was run with settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Species delimitation was first examined using single locus phylogenies. When significant conflict was not observed among the single locus phylogenies, then we concatenated two single-locus datasets (nrITS and nrLSU) into one multi-locus dataset using BioEdit v. 7.0.9 (Hall 1999). The combined two- locus (nrITS and nrLSU) dataset was phylogenetically analysed using Maximum Likelihood (ML). ML was performed using raxmlGUI 2.0 (Edler et al. 2021) with the GTRGAMMA substitution model. ML analysis was executed applying the rapid bootstrap algorithm with 1000 replicates to obtain nodal support values. Maximum Likelihood bootstrap (MLbs) values ≥ 70% are shown in the phylogenetic tree (Figs. 1, 4 & 7).

Phylogenetic inferences

The final combined (nrITS + nrLSU) datasets of our three species (*A. aurantialba*, *A. indovaginata* and *A. pseudohemibapha*) consisted of 61, 55 and 50 sequences with 1372, 1903 and 1117 characters respectively including gaps. Our three novel taxa are presented in the phylogenetic tree in bold red font (Figs. 1, 4 & 7).

In this study, our 2-locus (nrITS + nrLSU) phylogenetic analysis reveals that sequences derived from *Amanita aurantialba*, (voucher nos. AKA-31 and AKS-23) clustered with *Amanita pallidorosea*, a collection from China (voucher no. HKAS77349, HKAS82350, HKAS75483 and HKAS75786) and also forms a sister clade to *A. bisporigera* (voucher nos. RET 377-9) with a strong (MLbs = 97%) support (Fig. 1). Our second

species, *A. indovaginatae* (AGJH-036 and RGJ-22-02) clustered with *A. myrmeciae* a collection from Australia (voucher nos. RET 495 – 10, RET 473-8 and RET 687-7) clade with a strong (MLbs = 99%) support (Fig. 4) and this forms a sister clade with several other taxa viz., *Amanita justice* and *A. williamsiae*. On the other hand, our third species *A pseudohemibapha* (voucher nos. AGJH-062 and AGJH-078) are nested within the *A. hemibapha* lineage that included a collection from India and Thailand (voucher nos. TBGT 5872, RET-342-8, RMUKK02, RMUKH01, KY288023, RET 718-2 and TM 15–843). Within the *A. hemibapha* lineage, *A. pseudohemibapha* formed a significantly supported clade (MLbs = 89%) (Fig. 7).

Results

Taxonomy

Amanita aurantialba A. Kumar, Mehmood, A. Ghosh & Y.P. Sharma Figs. 2, 3.

MycoBank: MB 848552

GenBank: OQ553808 (nrITS, holotype), OQ561447 (nrLSU, holotype)

Typhification: INDIA. Jammu and Kashmir: Udhampur District, N 32^{o}43.030 E 75^{o}25.853 Elev. 1732 m, 25th August 2021, A. Kumar & K. Verma, AKA-31. (CAL 1922, holotype!).

Etymology: aurantialba (Lat.) referring to its orangish white Basidiomata of the type specimen

Holotypus: CAL 1922.

Diagnosis:Amanita aurantialba differs from the closest *Amanita* species by: pileus white (1A1) to orangish white (5A2) toward margin, brownish orange (5C4) over disc, limbate volva, apical white partial veil, broadly ellipsoid basidiospores 8–10 ×7–8 µm with (Qm =1.16), and its occurrence in the temperate mixed forest under *Quercus* sp.

Descriptions: Basidiomata medium-sized. Pileus 50–90 mm wide, convex to plano-convex pileus, finally becoming companulate to applanate with an umbo, often white, light orange (5A2) to pale orange (5C3), toward margin, brownish orange (5C4) over centre, smooth, sub-viscid, shinny, margin non-striated, pileus context 1–3 mm thick, thinning slowly toward margin, white, unchanging when cut or bruised. Universal veil remnants on pileus absent. Lamellae close to rather crowded (8-9 lamellae/10mm at margin, white or slightly pale orange (5A3) with maturity. Lamellulae attenuate, abundant, of various lengths. Stipe 120–150 × 15–25 mm (excluding bulb), subcylindrical, white (1A1) covered with finely white (1A1) fibrillose squamules. Partial veil subapical, thin, membranous, white skirt-like and persistent. Volva limbate, 35–40 × 30–35 mm thick, white to dirty white (1A1). Odour indistinct. Taste not recorded. Spore print white.

Basidiospores [60/2/3] (7–) 8–10 (–11) × (6–) 7–8 (–9) µm [Q= (1.11–) 1.14–1.28 (–1.42), Qm =1.16], broadly ellipsoid, amyloid, hyaline, thin-walled, smooth, hilar appendix up to 0.6 µm long; Lamellae edge

tissue sterile. Basidia 26–55 × 9–14 µm, clavate, 2-4-spored; sterigmata 3–5 µm long. Lamellae edge with clavate inflated cells (8–26 × 9–15 µm), colourless, frequent. Clamp connections not observed at the base of basidia. Hymenophoral trama bilateral, divergent. Mediostratum 80–130 µm wide, composed of abundant ellipsoid (32–82 × 13–33 µm); filamentous, undifferentiated hyphae 3–7 µm wide. Lateral stratum composed of ellipsoid to broadly ellipsoid cells (25–45 × 9–13 µm); filamentous undifferentiated hyphae 2–4 µm wide. Subhymenium 35–45 µm thick, basidia arising from subglobose to irregular inflated cells (11–17 × 9–12 µm). Pileipellis 75–135 µm thick, in two layers; upper layer 35–70 µm thick, radially arranged filamentous undifferentiated, thin-walled, colorless hyphae 3–5 µm wide; inner layer 40–65 µm thick; filamentous hyphae compactly arranged interwoven, thin-walled, hyaline 4–6 µm wide. Outer surface of volval remnants on stipe base filamentous undifferentiated hyphae 3–6 µm wide; inflated cells clavate to subglobose, 60–120 × 40–70 µm, thin-walled. Interior of volval remnants on stipe base composed of filamentous, undifferentiated hyphae 3–5 µm wide; inflated cells subglobose, cells 45–54 × 30–45 µm, with filamentous hyphae 4–7 µm wide. Partial veil filamentous undifferentiated hyphae 3–6 µm, wide, hyaline, slightly thick-walled, with pyriform or ellipsoid to broadly ellipsoid terminal cells, 30–60 × 11–25 µm. Clamp connections not observed.

Habitat & distribution: Solitary to scattered in temperate mixed forest dominated by *Quercus* species.

Known distribution: Only collected from north-western Himalaya of Jammu Province, India.

Additional specimens examined: INDIA. Jammu and Kashmir: Udhampur District, N 32^{o}43.030 E 75^{o}25.853 , Elev. 1732 m, 25th August 2021, A. Kumar & K. Verma, AKS-23.

Notes: Amanita aurantialba belonging to *Amanita* subg. *Amanitina* sect. *Phalloideae* is characterized by its typical pileus white to orangish white toward margin, brownish orange over disc, limbate volva, apical white partial veil, broadly ellipsoid basidiospores 8–10 ×7–8 µm with (Qm =1.16), and its occurrence in the temperate mixed forest under *Quercus* sp.

Morphologically, *Amanita aurantialba* shares some similarities with other *Amanita* species, namely *A. pallidorosea* P. Zhang & Zhu L. Yang, *A. subpallidorosea* Hai J. Li, *Amanita bisporigera* G.F. Atk., and *Amanita ocreata* Peck. However, *Amanita pallidorosea* differs by bearing a pinkish or reddish white to pale red colour pileus in the centre, a bilimbate volva, and globose to subglobose basidiospores (Zhang et al. 2010). *Amanita subpallidorosea* differs from *A. aurantialba* by its white pileus when young, which turns pale rose in the centre with age, lacerate squamules on the stipe, and large globose to subglobose basidiospores (Tulloss and Yang 2023). *Amanita bisporigera* from the USA has whitish, non-umbonate pileus and 2-spored basidia (Gilbert, 1940; Tulloss et al., 1995; Cui et al., 2018). *Amanita ocreata*, originally reported in the USA, can be distinguished from *A. aurantialba* by its large basidiomata with pinkish tan over the centre, fragile annulus, and subglobose basidiospores (range) (Jenkins 1986; Tulloss et al. 1995; Tulloss 2015). Based on phylogenetic inferences (Fig.1) the Indian *Amanita aurantialba* (OQ553808 and OQ561447) is clustered with other sequences of the sect. *Phalloideae* being sister to *A. pallidorosea* and *A. bisporigera*

Amanita indovaginata A. Ghosh, D. Chakr. & K. Verma Figs. 5, 6.

MycoBank: MB 848554

GenBank: OQ852757 (nrITS Holotype) and OQ852761 (nrITS); OQ847820 (nrLSU, holotype), OQ847821 (nrLSU).

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

*Typification:*INDIA. Jharkhnad: Sahibganj district, Partee Pahar, Ulghutu, Durgapur Panchayat, N 24°48.116' E 87°43.085', elev. 62 m, on ground, under *Shorea robusta* in tropical deciduous forests, 18th September 2022, A. Ghosh, AGJH-036 (CAL 1921, holotype!).

Diagnosis: *Amanita indovaginatae* differs from the closest species by: brownish grey pileus at the centre, faded chalky white pileus towards margins, inamyloid globose to broadly ellipsoid basidiospores measuring (11–14 × 10–12 µm), absence of clamp connections and its occurrence under *Shorea robusta* in tropical deciduous forests.

Descriptions: Basidiomata small to medium sized. Pileus 30–60 mm diam., initially convex, then becoming convex to plano-convex, depressed at centre, brownish grey (7–9C–D2) at the centre, gradually faded chalky white (1A1) towards margin, dry, viscid when moist, smooth, covered with greyish brown (7E3–4) minutely fibril, mostly lacking universal veil remnants; margin striate up to 18 mm, incurved. Pileus context chalky white (1A1), up to 2 mm thick, thinning towards margin, not bruising or staining. Lamellae free, crowded (8–14/10 mm at the pileus margin), chalky white (1A1), forked near the margin; lamellar edges concolorous; lamellulae truncate, plentiful. Stipe 45–120 × 6–12 mm, cylindrical to subclavate, chalky white (1A1) covered with white minute fibrils, context chalky white (1A1), hollow in center; basal bulb absents. Volva saccate, 16–50 × 10–27 mm, membranous, outer and inner surfaces chalky white to greyish white (1A1–B1). *Annulus* absent. Odour and taste not observed. Spore print white.

Basidiospores [60/3/2] (10.5–)11–14(–15) × (9.5–)10–12(–13) µm, Q = (1.0–)1.10–1.25(–1.35); Q = 1.16) globose to broadly ellipsoid, non-amyloid, hyaline, thin-walled, smooth, apiculus sublateral, up to 1 µm high; contents monoguttulate. Basidia (46–)48–60(–73) × (14–)15–17(–18) µm, 2- to 4-spored, thin-walled, colourless; sterigmata 3–7 µm long; clamp connections not observed at the base of basidia. Lamellar edge cells sterile, with inflated cells subglobose to pyriform, 25–45 × 12–40 µm, thin-walled, colourless, hyaline. Subhymenium w_{st}-near = 20–40 µm; w_{st}-far = 35–55 µm; basidia arising from subglobose to irregular shaped cells. Hymenophoral trama bilateral, divergent; = 30–50 µm wide, with inflated clavate cells, 20–40 × 8–12 µm; filamentous, undifferentiated hyphae (3–7) µm wide, colourless, hyaline. Pileipellis 95–115 µm thick, in two layers; gelatinized suprapellis 40–57 µm thick, composed of irregular filamentous, undifferentiated hyphae 4–6 µm wide, thin-walled, colourless, hyaline; nongelatinized subpellis 55–70 µm thick, composed of filamentous, undifferentiated hyphae 4–7 µm wide, radially arranged with yellowish brown intracellular pigments. Pileus trama filamentous

undifferentiated hyphae 3–8 µm wide; with broadly ellipsoid to ellipsoid cells (20–60 µm). Universal veil on stipe base with inflated cells globose to subglobose, 50–65 × 30–40 µm; filamentous, undifferentiated hyphae 5–11 µm wide. Stipe context longitudinally acrophysalidic; acrophysalides 102–204 × 20–28 µm; filamentous, undifferentiated thin walled, hyaline hyphae with 2–8 µm wide. Clamp connections absent in all tissues.

Habit & distribution: Solitary to scattered, on ground under *Shorea robusta* C. F. Gaertn. in tropical deciduous forests.

Additional specimens examined: INDIA. West Bengal: Uttar Dinajpur, Kaliyaganj, Dhamja, N 25°18'00" E 88°20'35.09", elev. 80 m, on ground, under *Shorea robusta* in tropical deciduous forests, 18.06.2022, A. Ghosh & D. Chakraborty, RGJ-22-02.

Notes: *Amanita indovaginata* is closely related to *Amanita glarea* S. Jabeen, M. Kiran & Sadiqullah. However, latter species has greyish brown to light grey pileus and globose to subglobose basidiospores (10.1–11.7 × 10.4–11) µm, with Q' = 1.08. Morphologically, *A. indovaginata* is also closely related to *A. vaginata* (Bull.) Lam except for the color of the pileus, which is bistre-grey to brownish grey when compared to *A. indovaginata* (Kibby 2003).

Morphologically, *Amanita indovaginata* is somewhat similar to some Indian species of the section *Vaginatae* and may be confused with these species viz., *Amanita cornelii* Mehmood et al.*A. dhakuriana* Mehmood et al. *Amanita subtropicana* Mehmood et al. However, *Amanita cornelii* is easily segregated by its pileus, which has crude and irregular pattern of brown radically oriented stripes, stipe covered by grey to brown fibrils and globose to subglobose basidiospores with lower Q value than the present undescribed taxon; Q' = 1.06 (Tibpromma et al. 2017). *Amanita indovaginata* differs from *A. dhakuriana* by its pileus, by its umbonate, greenish grey to yellowish grey pileus, and occurrence in the temperate mixed forest under *Abies pindrow* and *Quercus*, whereas, *A. emodotrygon* has greyish brown to olive campanulate pileus which is deeply grooved toward margin, crowded white lamellae that becomes pale yellowish with age, occurrence in a coniferous forest under *Pinus roxburghii* (Tibpromma et al. 2017). *Amanita subtropica* reported from same host can be easily separated from *Amanita indovaginata* by its olive brown to yellowish brown pileus, covered by felted, subconical, subpyramidal to warty universal veil remnants and broadly ellipsoid to ellipsoid basidiospores (Phookamsak et al. 2019).

Phylogenetically, the combined (nrITS and LSU) dataset places the new species, *Amanita indovaginata* (AGJH-015, AGJH-036) in a sister clade to *Amanita myrmeciae* (RET 495-10, RET 473-8 and RET 687-7), *Amanita justicei* (RET 697-5 and RET 691-2), *Amanita williamsiae* (RET 160-3, RET 478-10). However, A. *myrmeciae* can easily be segregated from the present species by its light brownish to brown pileus, and has subglobose basidiospores (9–11.2 × 8–10 µm) and associated with *Eucalyptus* sp. (Tulloss &Yang 2023). *Amanita justicei* differs by its yellowish to yellow-brown pileus with distinct umbo subglobose to broadly ellipsoid basidiospores with Qm = 1.16. (Tulloss &Yang 2023). *Amanita williamsiae* can be differentiated by having yellow color pileus with a prominent tannish umbo, and its occurrence in *Pinus* and *Quercus* (Tulloss &Yang 2023).

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Amanita pseudohemibapha A. Ghosh, Mehmood A. Kumar & Y.P. Sharma sp. nov. Figs. 8,9.

MycoBank: MB 848553

Genbank: OQ847828 (nrITS Holotype) and OQ847829 (nrITS); OQ847831 (nrLSU, holotype), OQ847830 (nrLSU).

Etymology: *pseudohemibapha* (Lat.) referring to its close resemblance with *Amanita hemibapha*

*Typification:*INDIA. Jharkhand: Sahibganj district, Borio block, Dhogada-Paharia burial ground forest, N 25°02'23.7" E 87°39'35.8", Elev. 110 m, on ground, under *Shorea robusta* in tropical deciduous forests, 24th September 2022, A. Ghosh, AGJH-062 (CAL 1920, holotype!).

Diagnosis: Amanita pseudohemibapha differs from the closest *Amanita* species by: orange to deep orange pileus over centre, yellow to vivid yellow toward margin, membranous annulus, saccate volva, broadly ellipsoid basidiospores (10–12 × 7–9 µm) with Qm =1.36 and abundant clamp connections in the basidiomata, its occurrence under *Shorea robusta* in subtropical mixed forests and nrITS and nrLSU sequence data

Descriptions: Basidiomata small to medium sized. Pileus 40–90 mm wide, convex at first then planoconvex to applanate, orange to deep orange (5A7–8) over centre, to vivid yellow (3A6–7) towards margin, slightly depressed at centre, shiny appearance; context 5–6 mm thick, yellowish white (2A2), thinning slowly toward margin, unchanging when cut or bruised but discolouring the pileus colour at maturity; margin tuberculate striate, striations up to 12 mm, forked near the margin. Lamellae free, yellowish white (3A2), close to crowded 8–11 lamellae/10 mm. Lamellulae truncate, of various lengths up to 6 mm thick. Stipe 60–65 × 7–8 mm, cylindrical to subclavate yellowish white scales all over the surface; context white, unchanging when cut or bruised, hollow. Partial veil apical, membranous, white, upto15mm long. Universal veil at stipe base saccate 15–30 × 12–16 mm, chalky white. Odour indistinct. Taste mild. Spore print white.

Basidiospores[80/3/3] (9–) 10–12 (–13) × (6.5–) 7–9 (–9.5) µm [Q= (1.22–) 1.25–1.44 (–1.57), Qm =1.36], broadly ellipsoid, amyloid, hyaline Basidia(35–) 40–45 (–50) × (9–)10–11.5(–12) µm, thin-walled, colourless, 2-4-spored; sterigmata up to 3–4 long; basal clamp present at the base of basidia. Lamella edge cells sterile; with inflated cells subglobose to broadly ellipsoid 12–20 × 15–25 µm, dominant, colourless, thin-walled. Subhymeniumup to 45–60 µm wide, composed of globose to subglobose cells up to 8 × 12 µm. Hymenophoral trama bilateral, divergent, filamentous, undifferentiated hyphae 4–14 µm wide, septate, thin-walled, hyaline; clamps present. Pileipellis 115–150 µm thick, slightly gelatinized, filamentous, undifferentiated hyphae 2–6 µm wide, branched, septate; clamps present. Pileus trama filamentous, undifferentiated hyphae 2–8 µm wide, septate, branched, thin-walled, hyaline, inflated cells broadly ellipsoid to elongated up to 212 × 36 µm, thin-walled, colourless, hyaline; clamp connections

present. Universal veil on stipe basefilamentous, undifferentiated hyphae 3–5 µm wide, thin-walled, colourless, hyaline, inflated cells globose to subglobose 30–50 × 25–42 µm, ellipsoid to elongated 16–40 × 10–16 µm; clamps present. Partial veilfilamentous, undifferentiated hyphae 4–8 µm wide, inflated cells dominant, globose to clavate up to 125–75 µm wide, colourless, thin-walled, hyaline. Stipe contextlongitudinally acrophysalidic; acrophysalides 116–230 × 20–36 µm; filamentous, undifferentiated hyphae 5–7 µm wide, hyaline. Clamp connections present in all tissue.

Habitat & distribution: Solitary to scattered, on ground under *Shorea robusta* C. F. Gaertn. in tropical deciduous forests.

Additional specimens examined: INDIA. West Bengal: Jhargram district, Lodhasuli, N 22°19'50", E 87°01'41", elev. 80 m, on ground, under *Shorea robusta* in tropical deciduous forests, 13th August 2020, A. Ghosh, AG 20-036; Jharkhand: Sahibganj district, Borio block, Dhogada-Paharia burial ground forest, N 25°02'23.7" E 87°39'35.8", elev. 110 m, on ground, under *Shorea robusta* in tropical deciduous forests, 25th September 2022, A. Ghosh, AGJH-078.

Notes: Amanita pseudohemibapha belonging to *Amanita* subg. *Amanitina* (E. J. Gilbert) E. J. Gilbert sect. *Caesare ae* is characterized by its small to medium sized basidiomata, orange to deep orange to vivid yellow pileus, broadly ellipsoid inamyloid basidiospores and its typical occurrence with Sal forests.

Morphologically, several species show close resembles to *Amanita pseudohemibapha* on the account of similar appearance such as *Amanita hemibapha* (Berk. & Broome) Sacc., *A. javanica* (Corner & Bas) T.oda, C.Tanaka & Tsuda, *Amanita aporema* Boedijn, *Amanita malayensis* L.P. Tang, Zhu L. Yang & S.S. Lee, as *A. caesareoides* Lj. N. Vassiljeva, *A. caesarea* (Scop: Fr.) Pers. and *Amanita similis* Boedijn. However, *Amanita hemibapha* was first reported from Ceylon (Berkeley & Broome 1870) and then from Japan (Imazeki & Hongo 1987; Oda et al. 1999), China (Yang 2005; 2015), India (Vrinda et al. 2005; Mehmood et al. 2018), differs from *A. pseudohemibapha* by its deep crimson pileus, yellow stipe covered with reddish patches, yellow annulus, and narrower basidiospores. *A. javanica* is also different from *A. pseudohemibapha* by its orange-yellow to ochre-yellow Pileus margin, orange-yellow stipe with orange-buff scales, absence of clamps in all tissues, and occurrence in small groups on the ground in dipterocarp forest. *Amanita aporema* differs from *A. pseudohemibapha* by its brown pileus with longer striae (more than 1/2R on the pileal margin) and globose basidiospores. *Amanita malayensis* can be distinguished by its margin with short striations, pileus at the centre that is apricot yellow to chrome yellow when young and becomes yellowish orange to saffron yellowish at maturity, ellipsoidal basidiospores, and its occurrence on soil in a park similarly. *A. caesareoides* wasoriginally described from Kamchatka Peninsula, Russia, and differs from present species by its large to ovate basidioma, pileus red to reddish orange, and its occurrence under *Quercus leucotrichophora*, *Rhododendron arboretum,* and *Pinus roxburghii*. *A. caesarea* a species of the Mediterranean Region, is also different from our present species by its bright orange-red to duller orange pileus, bright orange-red often becoming more or less pale at maturity, ellipsoid to elongate basidiospores, and its occurrence under *Castanea* and *Quercus* with *Pinus* and *Arbutus*. *Amanita similis* was originally reported from Indonesia and differs from current

species by its dark brown to olivaceous pileus, stipe yellow with orange scales, and its association with pine and oak (Boedijn 1951).

Phylogenetically, the new species, *Amanita pseudohemibapha,* is closely related to *Amanita hemibapha*. (Voucher no. RET-342-8, TBGT Pradeep 5872, TM 15-843, and RET 718-2). However, *A. hemibapha* is different by having yellowish orange to reddish yellow pileus with broadly ellipsoid to ellipsoid basidiospores and its occurrence under *Pinus roxbughii*. Both morphological as well as nrLSU and nrITS-based phylogenies clearly indicate the genetic dissimilarities of *A. pseudohemibapha* from these taxa from India and Thailand.

Statements & Declarations

Acknowledgments

The authors are grateful to the Director, Botanical Survey of India, Kolkata, and the Head, Department of Botany University of Jammu for providing facilities during the present study. AG is thankful to Science and Engineering Research Board (SERB), DST, Govt of India for providing the National Post-Doctoral Research Fellowship (files nos. PDF/2021/000183). AK also acknowledges the financial assistance received from University Grants Commission, New Delhi, in the form of Junior Research Fellowship

The authors confirm sole responsibility for the following: study conception, design, data collection, analysis and interpretation of results, and manuscript preparation.

Data availability

In this study, DNA sequences have been deposited in GenBank. Specimens were deposited at the Central National Herbarium (CAL)-Botanical Survey of India and Ministry of Environment, Botanical Garden, Shalimar, Howrah, West Bengal.

Competing interests

The authors do not have any relevant financial or non-financial interests to disclose

Funding

This study was financially supported by Science and Engineering Research Board (SERB), DST, Government of India, for providing the National Post-Doctoral Research Fellowship (file no. PDF/2021/000183) and the University Grants Commission (UGC), New Delhi, in the form of JRF (UGC.Ref.No.:667/CSIR-UGC NET JUNE 2018).

Authors' contributions

Aniket Ghosh and Anil Kumar collected the samples, conceived, designed, and used software for the construction of phylogeny. Anil Kumar prepared the materials, wrote the first draft of the manuscript and all contributors provided feedback on the prior draft. Tahir Mehmood made the line drawings and reviewed the article. Komal Verma contributed to the microscopic structures and interpretation of results. Dyutiparna Chakraborty prepared the outline of the manuscript and analyzed the results. Manoj Emanuel Hembrom helped in the data collection and construction of an idea for the manuscript. Yash Pal Sharma supervised and revised the article and suggested critical comments on it. All authors reviewed the results and approved the final version of the manuscript.

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Figures

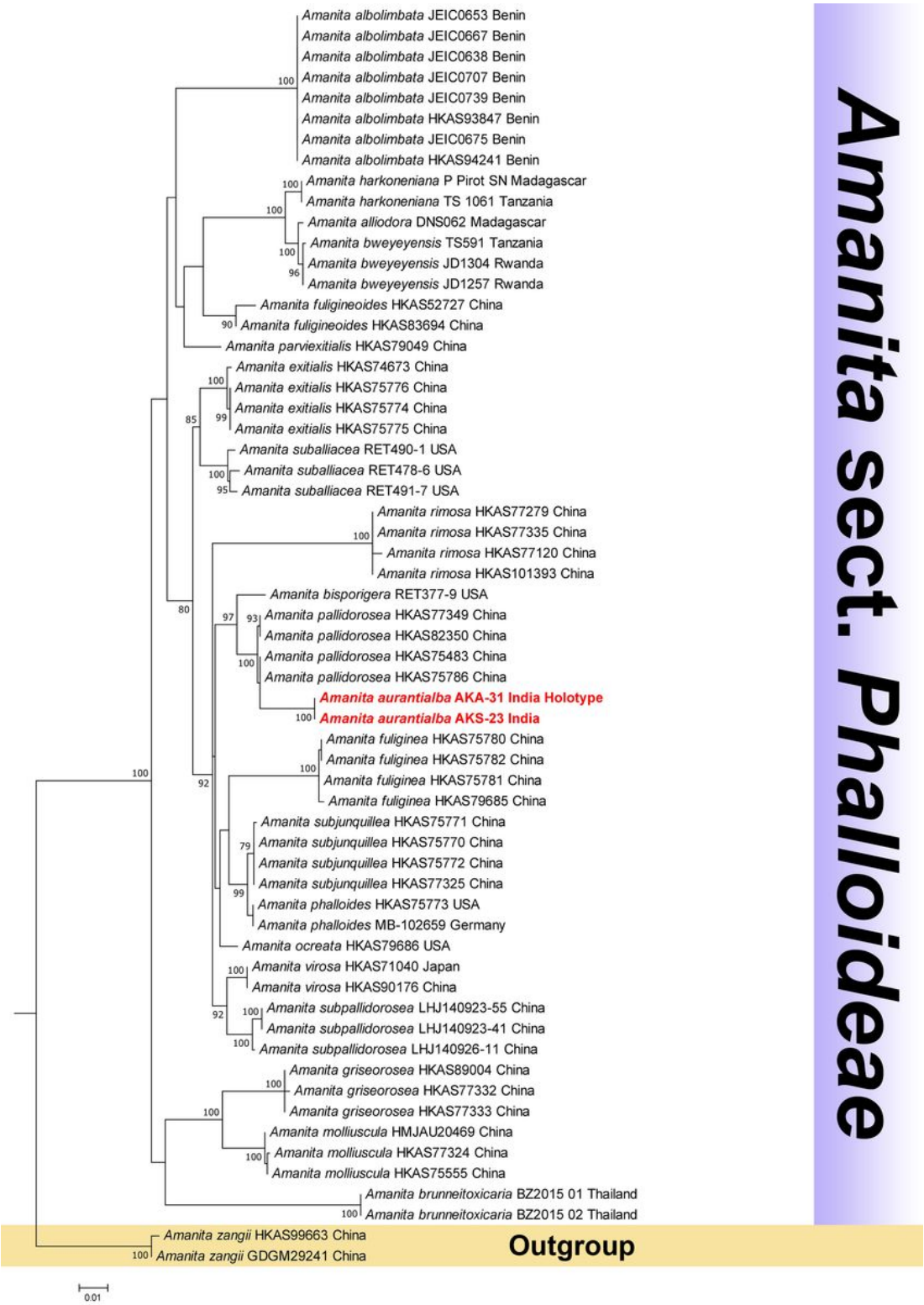

Figure 1

Phylogram generated by Maximum Likelihood analysis based on combined sequence data of nrITS and nrLSU for *Amanita aurantialba* and allied species. Maximum likelihood bootstrap support values (MLbs) ≥70% are shown on the above or below the branches at nodes. *A. aurantialba* is placed in bold red font to highlight its phylogenetic position in the tree.

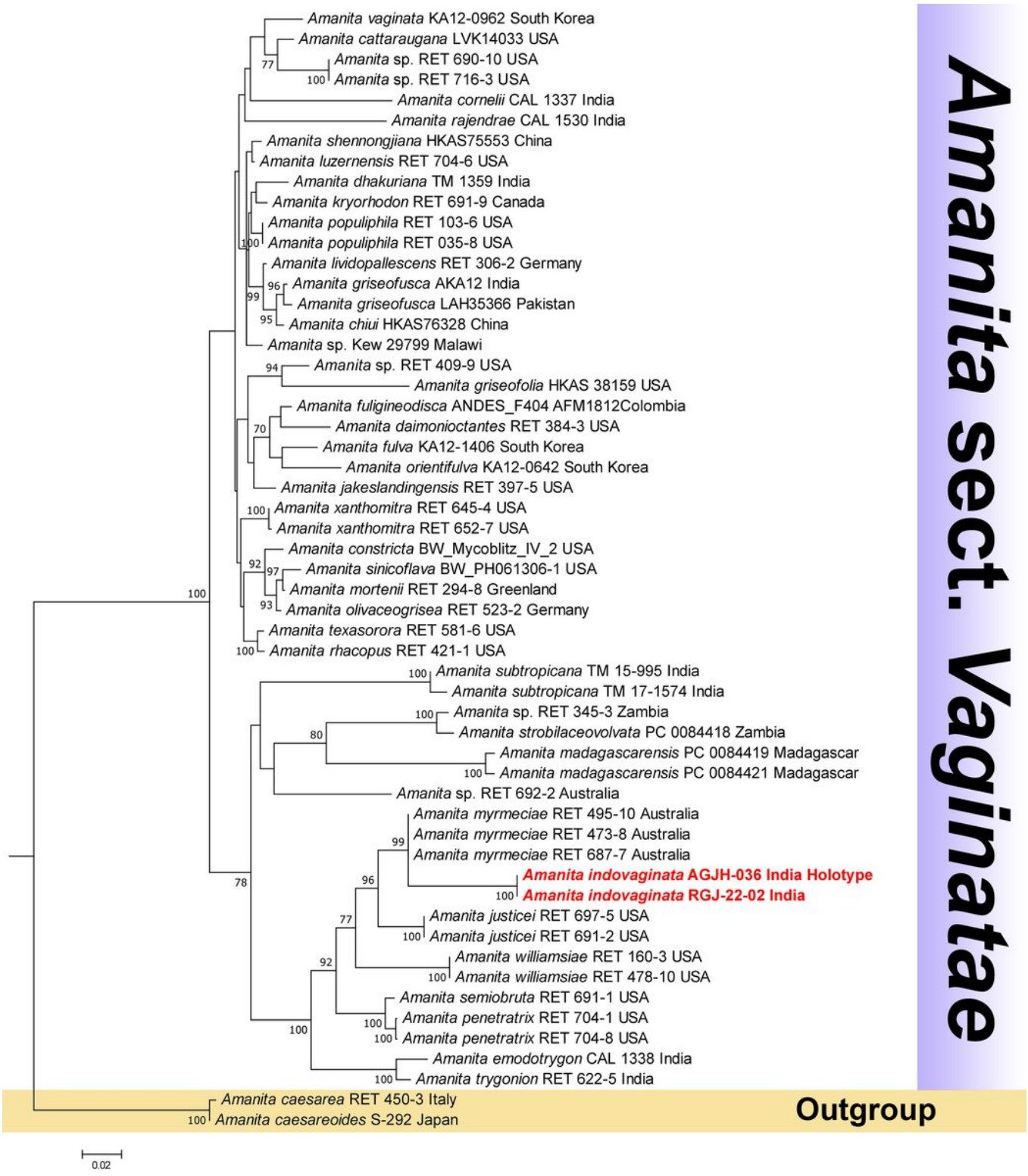

Figure 2

Amanita aurantialba sp. nov. (AKA-31 holotype). (a–c) Young and mature basidiomata in the field. (d) Basidia and elements of subhymenium (LM). (e) Light micrograph (LM) of Basidiospores. (f)) volval elements. Scale bars: a–c = 20 mm; d–f =10 µm.

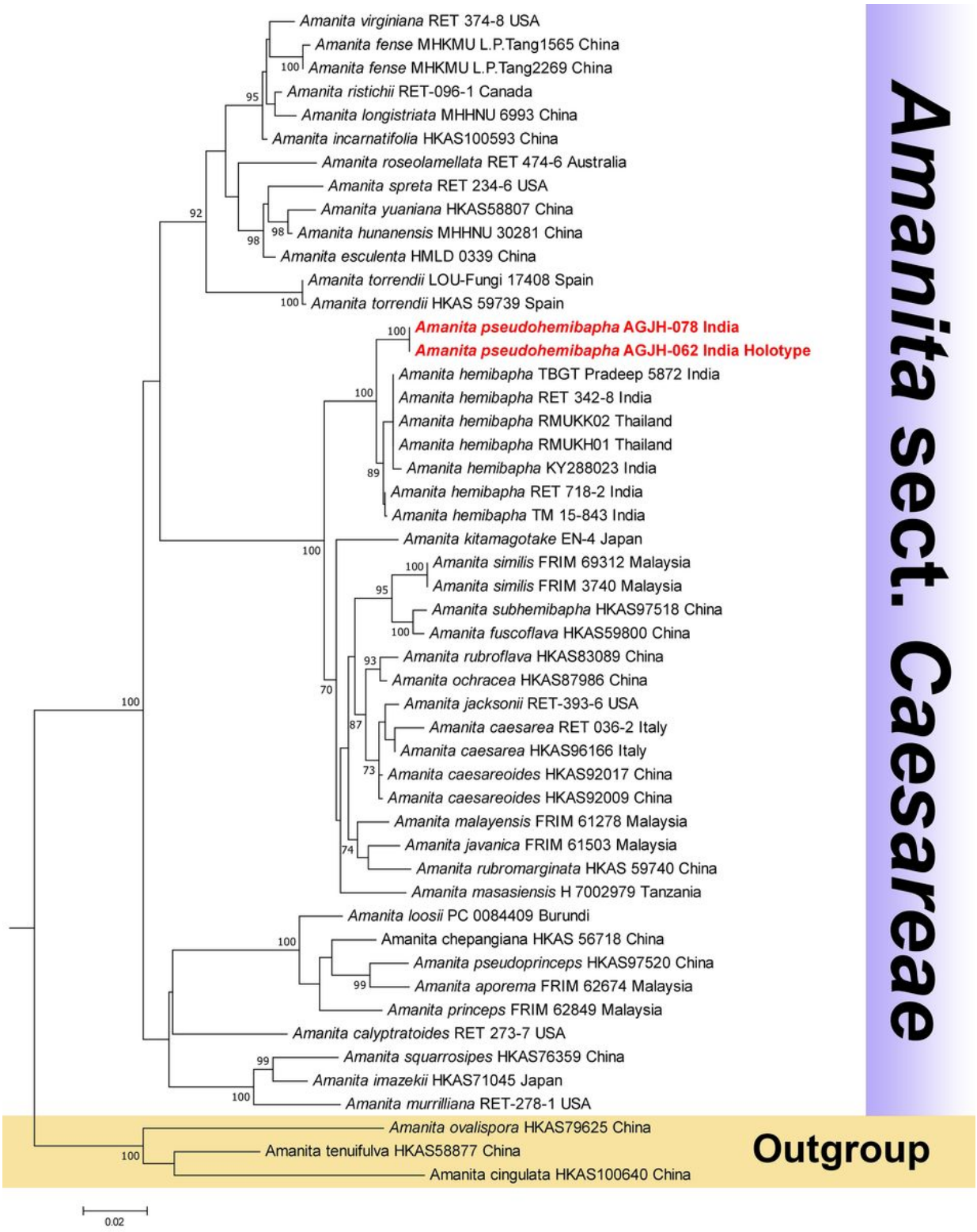

Figure 3

Microscopic features of *Amanita aurantialba sp. nov*. (AKA-31 holotype). (a) Basidia and elements of subhymenium. (b) Basidiospores. (c) volval elements. Scale bars: a-e =10 μm

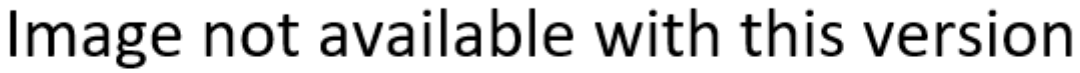

Figure 4

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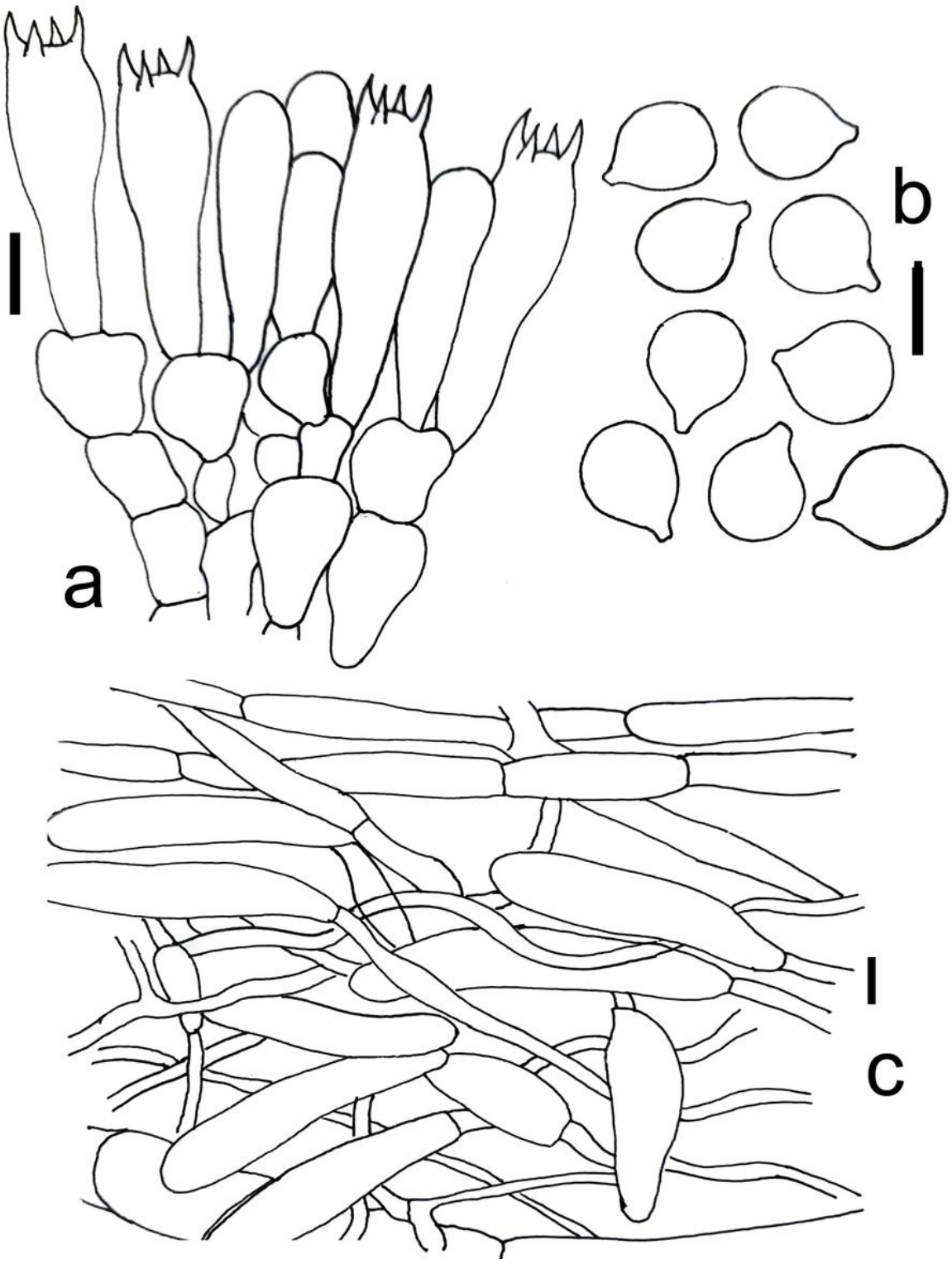

Figure 5

Amanita indovaginata sp. nov. (AGJH-015holotype). (a–b) Young and mature basidiomata in the field. (c-d) Basidia and elements of subhymenium (LM). (e-f) Light micrograph (LM) of Basidiospores in Melzer's reagent and phloxine (g-h) volval elements. Scale bars: a–b = 20 mm; c–h=10 µm.

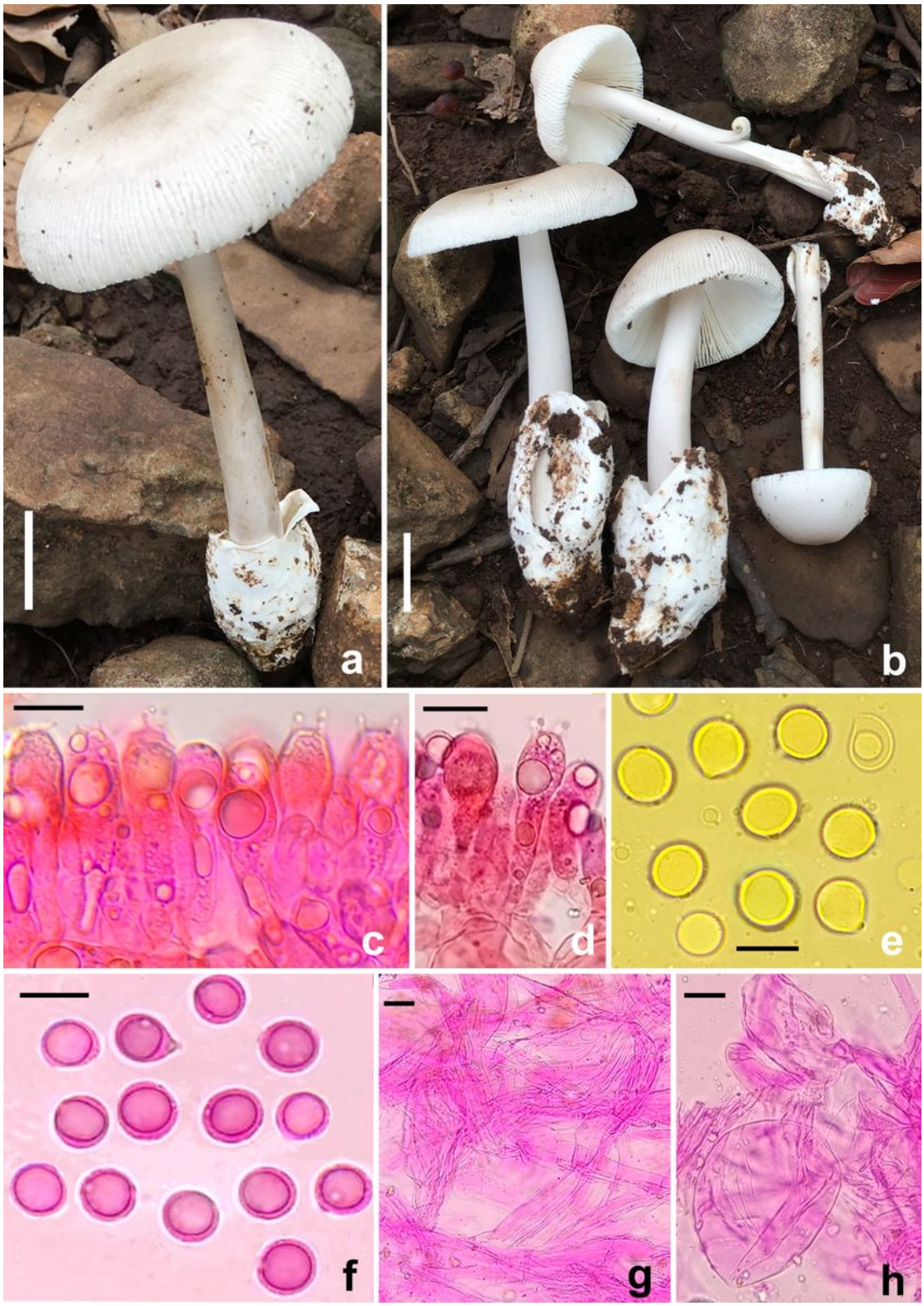

Figure 6

Microscopic features of *Amanita indovaginata sp. nov*. (AGJH-015 holotype). (a) Basidia and elements of subhymenium. (b) Basidiospores. (c)volval elements. Scale bars: a–c = 10 µm

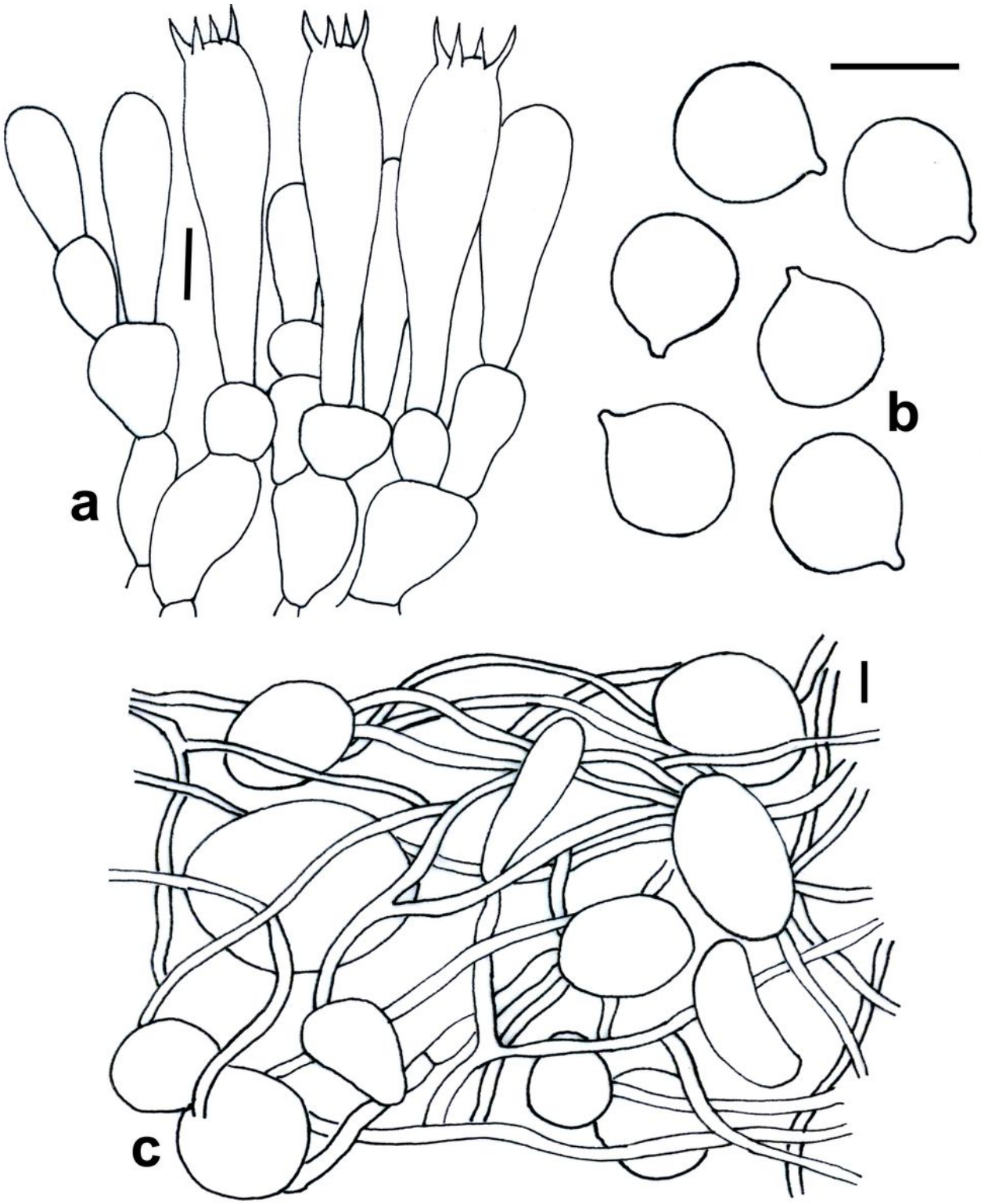

Figure 7

Phylogram generated by Maximum Likelihood analysis based on combined sequence data of nrITS and nrLSU for *Amanita pseudohemipbapha* and allied species. Maximum likelihood bootstrap support values (MLbs) ≥70% are shown on the above or below the branches at nodes. *A. pseudohemipbapha* is placed in bold red font to highlight its phylogenetic position in the tree.

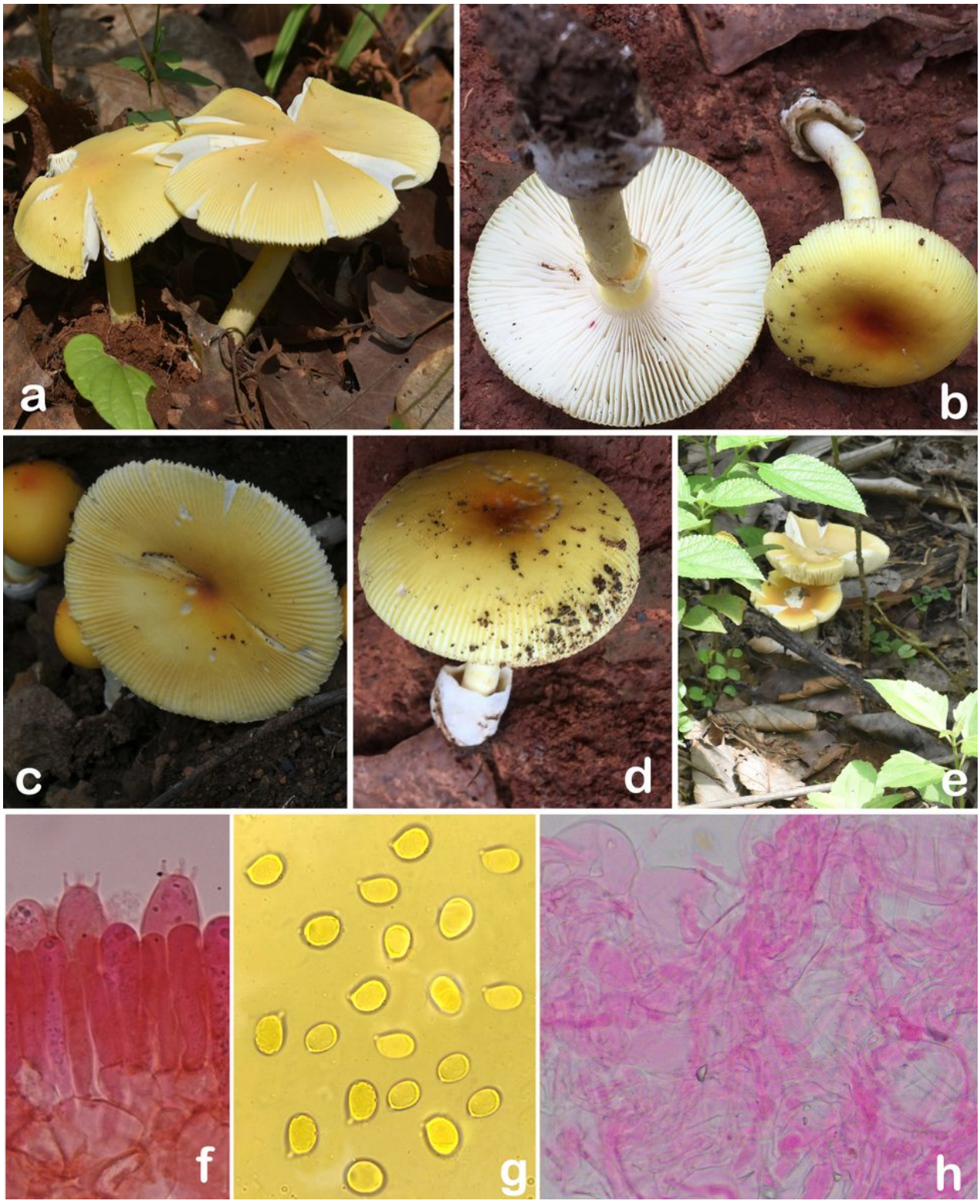

Figure 8

Amanita pseudohemibapha sp. nov. (AGJH-018-holotype). (a–e) Young and mature basidiomata in the field. (f) Basidia and elements of subhymenium (LM). (g) Light micrograph (LM) of Basidiospores. (h) volval elements. Scale bars: a–e = 20 mm; f–h =10 µm.

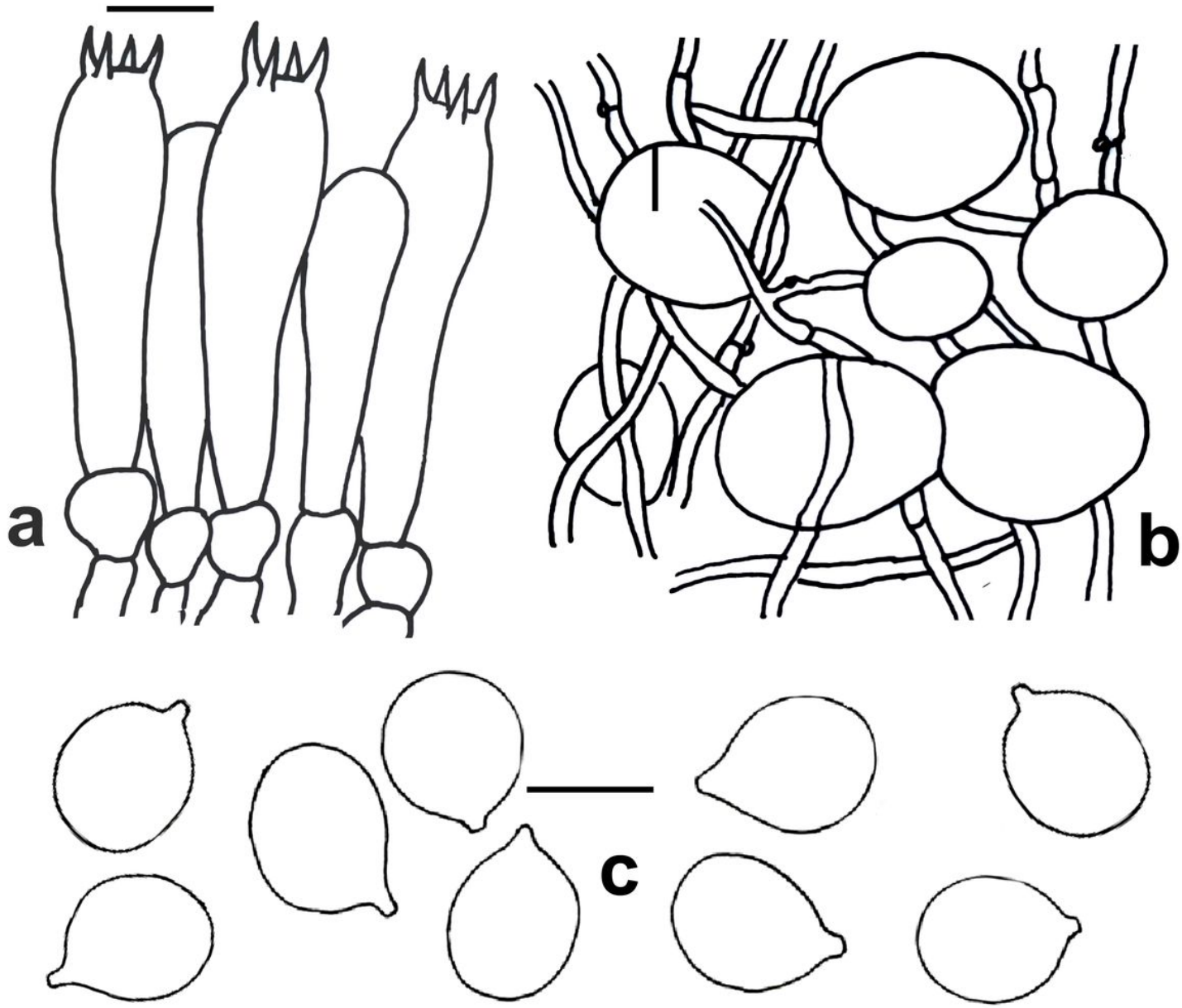

Figure 9

Microscopic features of *Amanita pseudohemibapha sp. nov*. (AGJH-018 - holotype). (a) Basidia and elements of subhymenium. (b) Basidiospores. (c). volval elements. Scale bars: a-c =10 µm

Supplementary Files

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- GraphicalAbstract.png