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Molecular phylogeny and morphology reveal a new species of genus *Rhodocybe sensu stricto* Maire (Entolomataceae; Agaricales) from Pakistan

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ABSTRACT
This study describes a new species of genus *Rhodocybe* (Fr.) Maire, *R. pakistanica* sp. nov., from Pakistan. The novel species can be recognized morphologically by convex to hemispherical or applanate to planoconvex and centrally depressed pileus which is dull reddish brown at the center and dull orange pale at margins having bright patches, light yellow to orange or light to gray lamellae, dull to pale reddish or light reddish gray to grayish red stipe having whitish fibrillose surface and basal mycelial threads. Microscopically, it is recognized by ovoid or oblong and cyanophilic basidiospores, lanceolate, subulate or ventricose-rostrate cheilo- and pleurocystidia with golden ochre or pale yellowish refringent contents, and the presence of gloeoplerous hyphae in lamellar and pileal trama having golden refractive contents. Macro and microscopic data and molecular phylogenetic analysis based on two nrDNA regions including internal transcribed spacers (ITS1-5.8S-ITS2 = ITS) and D1/D2 domain of large subunit (28S) indicate that the present taxon is a new species.

KEY WORDS
Pakistan,
Basidiomycota,
Agaricomycetes,
phylogenetic analysis,
morphology,
cystidia,
new species.

RÉSUMÉ
La phylogénie moléculaire et la morphologie révèlent une nouvelle espèce du genre Rhodocybe sensu stricto *Maire (Entolomataceae ; Agaricales) du Pakistan.*
Cette étude décrit une nouvelle espèce du genre *Rhodocybe* (Fr.) Maire, *R. pakistanica* sp. nov., du Pakistan. Cette nouvelle espèce se reconnaît morphologiquement à son pileus convexe à hémisphérique ou applati à planoconvexe et déprimé au centre, qui est brun rougeâtre terne au centre et orange pâle terne sur les bords avec des taches brillantes, des lamelles jaune clair à orange ou clair à gris, un stipe rougeâtre terne à pâle ou gris rougeâtre clair à rouge grisâtre ayant une surface fibrillaire blanchâtre et des fils mycéliens basaux. Au microscope, elle se reconnaît à ses basidiospores ovoïdes ou oblongues et cyanophiles, à ses chéilo- et pleurocystides lancéolées, subulées ou ventricosées à contenu réfringent ocre doré ou jaunâtre pâle, et à la présence d'hyphes gloéopléreuses dans le trama lamellaire et pileux à contenu réfringent doré. Les données macro et microscopiques et l'analyse phylogénétique moléculaire basée sur deux régions de l'ADNrn comprenant les espaceurs transcrits internes (ITS1-5.8S-ITS2 = ITS) et le domaine D1/D2 de la grande sous-unité (28S) indiquent que le présent taxon est une nouvelle espèce.

MOTS CLÉS
Pakistan,
Basidiomycota,
Agaricomycetes,
analyse phylogénétique,
morphologie,
cystidia,
espèce nouvelle.

INTRODUCTION

Rhodocybe proposed by Maire, is a genus of Entolomataceae, whose members are saprotrophic and grow typically on the ground between debris and infrequently on wood (Breitenbach & Kränzlin 1995; Knudsen & Vesterholt 2008). The genus was traditionally divided into seven sections viz., *Claudopodes* Singer ex T.J.Baroni, *Crepidotoides* Singer, *Decurrentes* (Konrad & Maubl.) Singer, *Rhodocybe* Maire, *Rhodophana* (Kühner) Singer, *Rufobrunnea* T.J.Baroni, and *Tomentosi* T.J.Baroni (Baroni 1981). In the multigene molecular phylogenetic analysis conducted by Kluting *et al.* (2014), the genera *Clitopilopsis* Maire and *Rhodophana* were resurrected and *Clitocella* Kluting, T.J.Baroni & Bergemann described as a new genus. Therefore, the genus *Rhodocybe sensu lato* includes *Rhodocybe sensu stricto*, *Clitocella*, *Clitopilopsis*, and *Rhodophana*. The distinguishing features of *Rhodocybe* comprise pleurotoid, collybioid, mycenoid, clitocyboid or tricholomatoid basidiomata with varying coloration, adnexed to adnate or decurrent lamellae, pink or flesh-pink spore prints, basidiospores having a thin wall, uniformly cyanophilic, minutely angular with 6-12 facets in the polar view, randomly bumpy to pustulate ornamentation, presence or absence of cystidia and absence of clamp connections in all tissues (Baroni 1981; Kluting *et al.* 2014; Vizzini *et al.* 2018; Sesli 2021).

Until now, only two species of the genus *Rhodocybe sensu stricto* were recorded from Pakistan, viz., *R. subgilva* (Berk. & Broome) Pegler and *R. truncata* (Schaeff.) Singer (Ahmad *et al.* 1997; Sultana *et al.* 2011; Aman *et al.* 2022). Recent morphological and molecular studies in Noorpur Thal, Khushab district on macrofungi indicate that this region is highly diverse in macrofungal community (Haqnawaz *et al.* 2023; Izhar *et al.* 2023a, b; Khan *et al.* 2023a, b). The present work describes a new species of genus *Rhodocybe* from this fertile region of Pakistan based on morphological and molecular analysis encompassing ITS and 28S regions of nrDNA.

MATERIAL AND METHODS

Sampling site

Sampling was done by collecting basidiomata from the region of Peelowains located in tehsil Noorpur Thal of Khushab district during the rainy season in July and August 2022. The word Khushab has been derived from two Persian words “Khush” and “Aab” which means sweet or tasty water. The distinctive landscape of district Khushab is varied, consisting of agriculture lowlands, sand dunes, mountains, and lakes. Noorpur Thal is one of four tehsils of the Khushab district, a part of the Thal Desert of Punjab province. It is situated between 31°98’33”N and 71°91’67”E at an elevation of 185 meters a.s.l. The temperatures range between 3 to 8°C in winter (December to February) and 32 to 40°C in summer (May to July). The average annual rainfall varies from 185 mm to 300 mm. Major tree flora of the area includes *Albizia lebbeck* (L.) Benth, *Azadirachta indica* A.Juss., *Dalbergia sissoo* Roxb., *Eucalyptus camaldulensis* Dehnh., *Tamarix aphylla* (L.) Karst., *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb., and *Ziziphus nummularia* (Burm.f.) Wight & Arn., etc. The main cultivated crops in this area are *Cicer arietinum* L., *Cyamopsis tetragonoloba* L., and *Triticum aestivum* L. (Chaudhari *et al.* 2014; Shaheen *et al.* 2017; Khan *et al.* 2023a, b).

Morphological protocols

The samples were taken and photographed along with labeled tags, then brought to the Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab Lahore, and dried with a fan heater. Morphological characteristics of basidiomata such as size, shape, color, etc. were noted and measurements of macroscopic structures were taken (Vellinga & Noordeloos 2001). The samples were deposited in the Lahore Herbarium (LAH), Institute of Botany, University of the Punjab Lahore. For the microscopic study, 5% KOH (potassium hydroxide) was used for the rehydration of gills, pileus, and stipe tissues. Then Congo red was used for staining these tissues; whereas, Melzer’s reagent was used to test the amyloid reaction of the basidiospores. Microscopic structures such as basidia, basidiospores, and elements of pileus and stipe were observed under the microscope (CXRII, Labomed, Labo America Inc., United States) with HDCE-X5 attached camera, and measurements of microscopic structures were taken with ScopeImage 9.0 (×5) software (Meiji Techno, Saitama, Japan). The representations [n/m/p] show that the ‘n’ number of basidiospores measured from ‘m’ basidiomata of ‘p’ collections.

Abbreviations

For all of the spores analyzed, the following acronyms are used:

avl	average length;
avw	average width;
Q	length/width;
avQ	average Q value.

Molecular phylogenetic protocols

DNA from dried samples was extracted using the modified CTAB method (Zhao *et al.* 2011). For PCR amplification of ITS region, ITS1F (forward primer) and ITS4 (reverse primer) were used whereas to amplify 28S region, LROR (forward primer) and LR5 (reverse primer) were used (Vilgalys & Hester 1990; White *et al.* 1990; Gardes & Bruns 1993). PCR for ITS region was performed with initial denaturation at 94°C for four minutes, 40 cycles at 94°C for one minute, 55°C for one minute, 72°C for one minute, and a final extension at 72°C for ten minutes. While for 28S region, it was carried out with initial denaturation at 94°C for two minutes, 35 cycles at 94°C for one minute, 52°C for one minute, 72°C for one minute, and a final extension at 72°C for seven minutes. Then agarose gel electrophoresis was done to visualize the PCR products. Finally, the sequencing of PCR products was done from Tsingke, Beijing, China. The consensus sequences of forward and reverse primer read of ITS and 28S regions were generated by BioEdit software (v.7.2.5) (Hall 1999). The consensus sequences were BLAST searched in the NCBI database. The sequences that showed maximum identity with our sequence were downloaded. The sequences of previously

described species related to our species were also retrieved from the GenBank and UNITE database (Sesli & Vizzini 2017; Baroni *et al.* 2020; Dutta *et al.* 2021; Xavier *et al.* 2022). These ITS and 28S sequences were aligned separately using online alignment multiple sequence comparison by log expectation (MUSCLE) program (Edgar 2004) and then edited manually in BioEdit (Hall 1999). Maximum likelihood (ML) method was used to conduct Phylogenetic inference. The final phylogenetic trees of ITS and 28S sequences were generated using CIPRES Science Gateway online (Miller *et al.* 2010) employing RAxML-HPC v.8. (Stamatakis 2014). The phylogenetic trees were displayed in FigTree v.1.4.3 (Rambaut 2014). As there were conflicts in the topology of 28S sequence-based tree in comparison to the ITS based tree, the ITS and 28S alignments were concatenated end-to-end for each organism to create a supermatrix. This merged file was then exported in BioEdit and some manual editing was done such as positions with gaps were excluded, and non-conserved or flanking regions were removed. This refined concatenated dataset was used to construct a combined ITS-28S based phylogenetic tree using the method explained above.

Our ITS-based phylogenetic tree consisted of 59 nucleotide sequences as ingroup representing the taxa belonging to genera viz. *Rhodocybe*, *Clitopilus*, *Clitopilopsis*, *Clitocella*, *Rhodophana*, and *Entoloma*. *Calocybe carnea* (AF357028) and *Lyophyllum decastes* (AF357059), both belonging to Lyophyllaceae were used as outgroup taxa because this family is sister to Entolomataceae (Matheny *et al.* 2006). The combined ITS-28S sequence-based phylogram comprises 59 ITS sequences and 39 LSU sequences including two outgroup sequences (Table 1).

RESULTS

Phylogenetic analysis

The final aligned ITS region dataset comprised a total of 858 characters of which 235 were conserved, 587 were variable, 458 were parsimony informative and 112 were singletons. In the final ITS-based phylogenetic tree, three sequences of *Rhodocybe pakistanica* sp. nov. formed a separate sister subclade from other sequences of *Rhodocybe* (Fig. 1). The final combined ITS-28S dataset comprised a total of 1770 bases. The newly generated sequences clustered in a sister subclade with strong bootstrap support from other taxa of *Rhodocybe* (Fig. 2).

Family Entolomataceae Kotl. & Pouzar
Genus *Rhodocybe* (Fr.) Maire

Rhodocybe pakistanica sp. nov.
(Figs 3-5)

The species is mainly characterized by centrally depressed and dull reddish brown or dull orange pileus with bright patches, light yellow to orange lamellae, light reddish gray and fibrillose stipe, ovoid or oblong basidiospores, lanceolate, subulate or ventricose-rostrate cheilo and pleurocystidia, and presence of gloeoplerous hyphae in pileal and hymenial trama.

Type material. — **Pakistan** • Punjab Province, District Khushab, Noorpur Thal, Peelowains, on moist sandy soil with litter; 32°01'22.4"N, 71°59'32.4"E; 191 m a.s.l.; 01.VIII.2022; *Zaman Khan & Junaid Khan P-154*; holotype: LAH37947; GenBank: OR606543 (ITS), OR606541 (28S).

Additional material examined. — **Pakistan** • Punjab Province, District Khushab, Noorpur Thal, Peelowains, on moist sandy soil near *Ziziphus nummularia* (Burm.f.) Wight & Arn.; 31°59'17.36"N, 71°59'25.86"E; 191 m a.s.l.; 30.VII.2022; *Zaman Khan & Junaid Khan P-146*; LAH37948; GenBank: OR606544 (ITS), OR606542 (28S) • *ibid.*; 02.VIII.2022; *Zaman Khan & Junaid Khan P-171*; LAH37949; GenBank: OR606545 (ITS).

Etymology. — The specific epithet 'pakistanica' refers to the type locality of species.

Habit, habitat, and distribution. — Saprotrophic, solitary, terrestrial, on moist, sandy soil with litter in forest, near *Dalbergia sissoo*, and *Ziziphus nummularia*. Presently only known from Punjab, Pakistan.

MycoBank. — MB850258.

Description

Basidiomata

18-30 mm in length, small-sized. Pileus 7-28 mm diam., surface dull reddish brown (5YR 4/4, 5/4), at center becoming dull orange (7.5YR 6/4) or pale (5YR 8/3) towards periphery, with bright patches (7.5YR 5/6) scattering towards margins when mature, initially convex or hemispherical, depressed at center, becoming applanate or planoconvex and slightly depressed with age, margins decurved or plane at initial stage, uplifted or flaring upon maturity, undulate, entire.

Lamellae

Light yellow (10YR 8/3) to orange (10YR 8/4) or light (5YR 8/1) to gray (5YR 8/2), subdecurrent to decurrent, arcuate, broad or ventricose, brittle, entire or undate margins, lamellulae present in four tiers.

Stipe

15-28 × 2-4 mm, central, cylindrical or slightly tapering downwards, dull (2.5YR 6/3) to pale reddish (2.5 YR 7/3) or light reddish gray (7.5R 7/1) to grayish red (7.5R 6/2), with whitish fibrils on the surface, with rhizomorphs and whitish mycelial threads.

Context

Solid, pale reddish. Odor and taste not recorded.

Basidiospores

[50/3/3], 5.3-7 × 3.8-4.6 µm, avl × avw = 5.9 × 4.2 µm, Q = 1.35-1.52, avQ = 1.42, ovoid or oblong, sometimes ellipsoid, slightly undulate-pustulate, cyanophilic, inamyloid, non-dextrinoid, with prominent apiculus.

Basidia

21-26 × 6-8 µm, narrowly clavate to clavate, cyanophilic, with refractive contents in 5% KOH, thin-walled, two to four spored, sterigmata up to 4.4 µm in length.

TABLE 1. — Voucher/strain/isolate, country, GenBank/UNITE accession number, and reference for the specimens included in nrITS and combined ITS-28S phylogenetic analysis. The sequences produced during this study are shown in **bold font**.

Taxon name	Voucher/strain/isolate	GenBank/UNITE accession no.		Country	Reference
		nrITS	28S		
Calocybe carnea (outgroup)	CBS552.50	AF357028	AF223178	–	Hofstetter *et al.* 2002
Clitocella fallax	CBS605.79	AF357018	AF223165	–	Hofstetter *et al.* 2002
Clitocella fallax	CBS129.63	AF357017	AF223166	–	Hofstetter *et al.* 2002
Clitocella mundula	TJB7599	DQ494694	AY700182	–	Matheny *et al.* 2006
Clitocella mundula	HMJAU 7275	MN061331	MN065723	China	Jian *et al.* 2020
Clitocella orientalis	KUN-HKAS 75664 (Liu53)	MN061332	MN065726	China	Jian *et al.* 2020
Clitocella orientalis	KUN-HKAS 75548 (Cai794)	MN061333	MN065727	China	Jian *et al.* 2020
Clitopilopsis albida	KUN-HKAS 104519 (JSP224)	MN061335	MN065730	China	Jian *et al.* 2020
Clitopilopsis albida	KUN-HKAS 104520 (JSP225)	MN061336	MN065731	China	Jian *et al.* 2020
Clitopilopsis hirneola	MEN 199956	KC710132	GQ289211	–	Morgado *et al.* 2013
Clitopilopsis hirneola	CBS 126.46	MH856141	AF223163	France	Vu *et al.* 2019
Clitopilus passeckerianus	CBS 299.35	MH855682	MH867198	Austria	Vu *et al.* 2019
Clitopilus scyphoides	KRAM5	MN744420	MN744422	India	-
Clitopilus scyphoides	CBS 127.47	MH856181	MH867707	France	Vu *et al.* 2019
Clitopilus sinoapalus	KUN-HKAS 77037 (Wu865)	MN061321	MN065713	China	Jian *et al.* 2020
Clitopilus sinoapalus	KUN-HKAS 101191 (Yang6002)	MN061322	MN065711	China	Jian *et al.* 2020
Clitopilus umbilicatus	KUN-HKAS 80310 (Han79)	MN061324	MN065716	China	Jian *et al.* 2020
Clitopilus umbilicatus	KUN-HKAS 104509 (Wu2506)	MN061327	MN065719	China	Jian *et al.* 2020
Entoloma caccabus	MEN 200324	KC710063	GQ289155	–	Morgado *et al.* 2013
Entoloma conferendum	MEN 200330	KC710055	KC710133	Slovakia	Morgado *et al.* 2013
Entoloma serrulatum	LE254361	KC898447	KC898501	Russia	Morozova *et al.* 2014
Entoloma sp.	Sulzbacher 433/1-D-1	LT594987	–	Brazil	-
Entoloma tjallingiorum	LE254318	KC898411	KC898510	Russia	Morozova *et al.* 2014
Lyophyllum decastes (outgroup)	JM87/16(T1)	AF357059	AF042583	–	Hofstetter *et al.* 2002
Rhodocybe asanii	KATO:Fungi:3657	KX834265	–	Türkiye	Sesli & Vizzini 2017
Rhodocybe asanii	KATO:Fungi:3659	NR_154442	NG060176	Türkiye	Sesli & Vizzini 2017
Rhodocybe asyae	KATO:Fungi:3653	KX834268	–	Türkiye	Sesli & Vizzini 2017
Rhodocybe asyae	KATO:Fungi:3640	NR_154443	NG060177	Türkiye	Sesli & Vizzini 2017
Rhodocybe brunneoaurantiaca	CUH AM720	MW023201	MW023223	India: West Bengal	Dutta *et al.* 2021
Rhodocybe brunneoaurantiaca	CAL 1825	MW031906	MW031916	India: West Bengal	Dutta *et al.* 2021
Rhodocybe caelata	JVG 1070904-2	KU862855	–	Spain	Vizzini *et al.* 2016
Rhodocybe formosa	Herb. B. Picillo 12/208	KU862858	–	Italy	Vizzini *et al.* 2016
Rhodocybe formosa	LIP JVG 1061015	KU862856	–	Spain	Vizzini *et al.* 2016
Rhodocybe fumanellii	MCVE:29550	NR_166243	NG068264	Italy	Vizzini *et al.* 2018
Rhodocybe fusipes	DLK 587	MN306210	–	Brazil	Silva-Filho *et al.* 2020
Rhodocybe fusipes	DLK 298	MN306209	–	Brazil	Silva-Filho *et al.* 2020
Rhodocybe incarnata	REH5369	MT254071	–	Venezuela	Silva-Filho *et al.* 2020
Rhodocybe luteobrunnea	CAL:1322	NR_154434	NG060167	India	–
Rhodocybe matesina	MCVE:29261	KY629962	KY629964	Italy	–
Rhodocybe matesina	MCVE:29262	NR_154455	NG060184	Italy	–
Rhodocybe mellea	JBSD127402	MN784993	–	Dominican Republic	Baroni *et al.* 2020
Rhodocybe mellea	CORT013885	MN784992	–	Dominican Republic	Baroni *et al.* 2020
Rhodocybe mellea	NYBG815044	MN784995	–	Costa Rica	Baroni *et al.* 2020
Rhodocybe mellea	CORT014470	MN784994	–	Belize	Baroni *et al.* 2020
Rhodocybe mellea var. *depressa*	F. Wartchow 08-2019	MT408926	OL687341	Brazil	Xavier *et al.* 2022
Rhodocybe minutispora	LIP JVG 1071101	KU862860	–	Spain	Vizzini *et al.* 2016
***Rhodocybe pakistanica* sp. nov.**	**LAH37948**	**OR606544**	**OR606542**	**Pakistan**	**This study**
***Rhodocybe pakistanica* sp. nov.**	**LAH37949**	**OR606545**	**–**	**Pakistan**	**This study**
***Rhodocybe pakistanica* sp. nov. (holotype)**	**LAH37947**	**OR606543**	**OR606541**	**Pakistan**	**This study**
Rhodocybe pallidogrisea	CORT 013944	KX271752	–	Australia	–
Rhodocybe roseiavellanea	PBM4056 (TENN)	MF686525	–	United States	–
Rhodocybe rubrobrunnea	CAL 1387	KX951452	–	India	–
Rhodocybe sp.	Sulzbacher 340 (UFRN-fungos 2557)	LT594979	–	Brazil	Sulzbacher *et al.* 2017
Rhodocybe sp.	Sulzbacher 413 (UFRN-fungos 2062)	LT594984	–	Brazil	Sulzbacher *et al.* 2017
Rhodocybe truncata	CBS482/50	EF421110	AF223167	–	–
Rhodocybe tugrulii	IMG-7316	MG050105	MG050111	United States	–
Rhodocybe tugrulii	KATO:Fungi:3340	NR_154436	NG060175	Türkiye	–
Rhodophana griseobrunnea	LUG 19799	MT580804	MT580803	France	–
Rhodophana nitellina	TU106939	UDB015654	–	Estonia	–
Rhodophana nitellina	TU106585	UDB011813	–	Estonia	–
Rhodophana squamulosa	CAL:1262	KT180329	NG060152	India	Raj *et al.* 2016

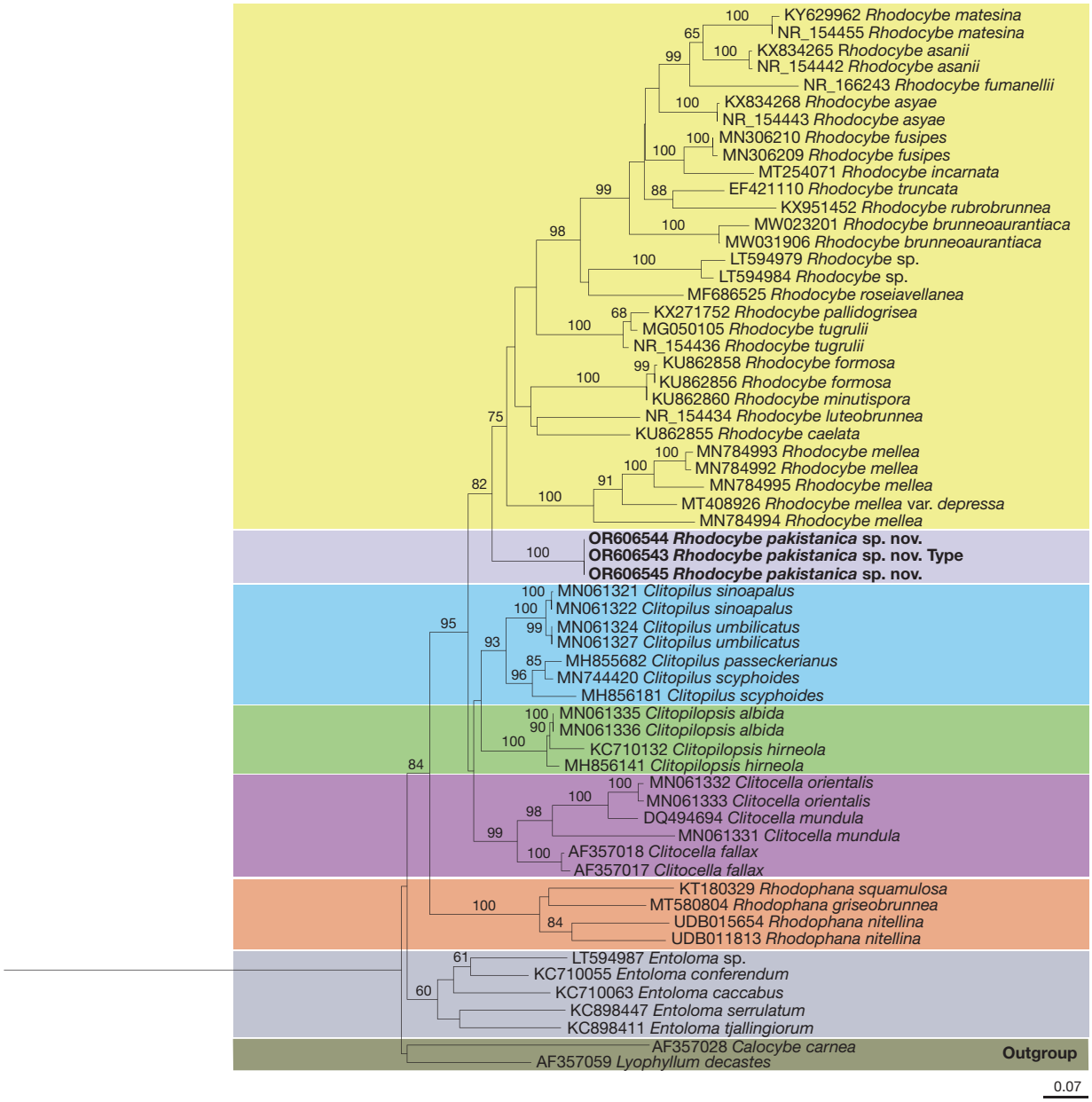

FIG. 1. — Maximum likelihood phylogenetic tree of sequences of Entolomataceae Kotl. & Pouzar, resulting from the analysis of nrITS sequences; maximum likelihood bootstrap BT support values greater than 50% are written above the nodes; new species *Rhodocybe pakistanica* sp. nov. is indicated in **bold font**.

Cheilo- and pleurocystidia
Rarely present, 24-37 × 4-7 µm, lanceolate, subulate, ventricose-rostrate, sometimes fusiform, cylindrical or clavate, with golden ochre or pale yellowish refractive contents in 5% KOH.

Lamellar trama
Consisting of 5.5-12.5 µm broad, regular, cylindrical, interwoven hyphae, hyaline in 5% KOH.

Gloeoplerous hyphae in hymenial and pileal trama
3.2-7.7 µm broad, cylindrical, elongated, with narrow endings, incrusted, golden refringent in 5% KOH.

Pileipellis
A cutis, consisting of 2.4-6.7 µm broad, cylindrical or elongate, thin-walled hyphae, brownish in 5% KOH.

Stipitipellis
A cutis, consisting of 3.2-8.8 µm broad, cylindrical, parallel, rarely branched hyphae, with clavate or narrowly fusiform ending elements, hyaline in 5% KOH.

Caulocystidia
Absent.

Clamp connections
Absent in all tissues.

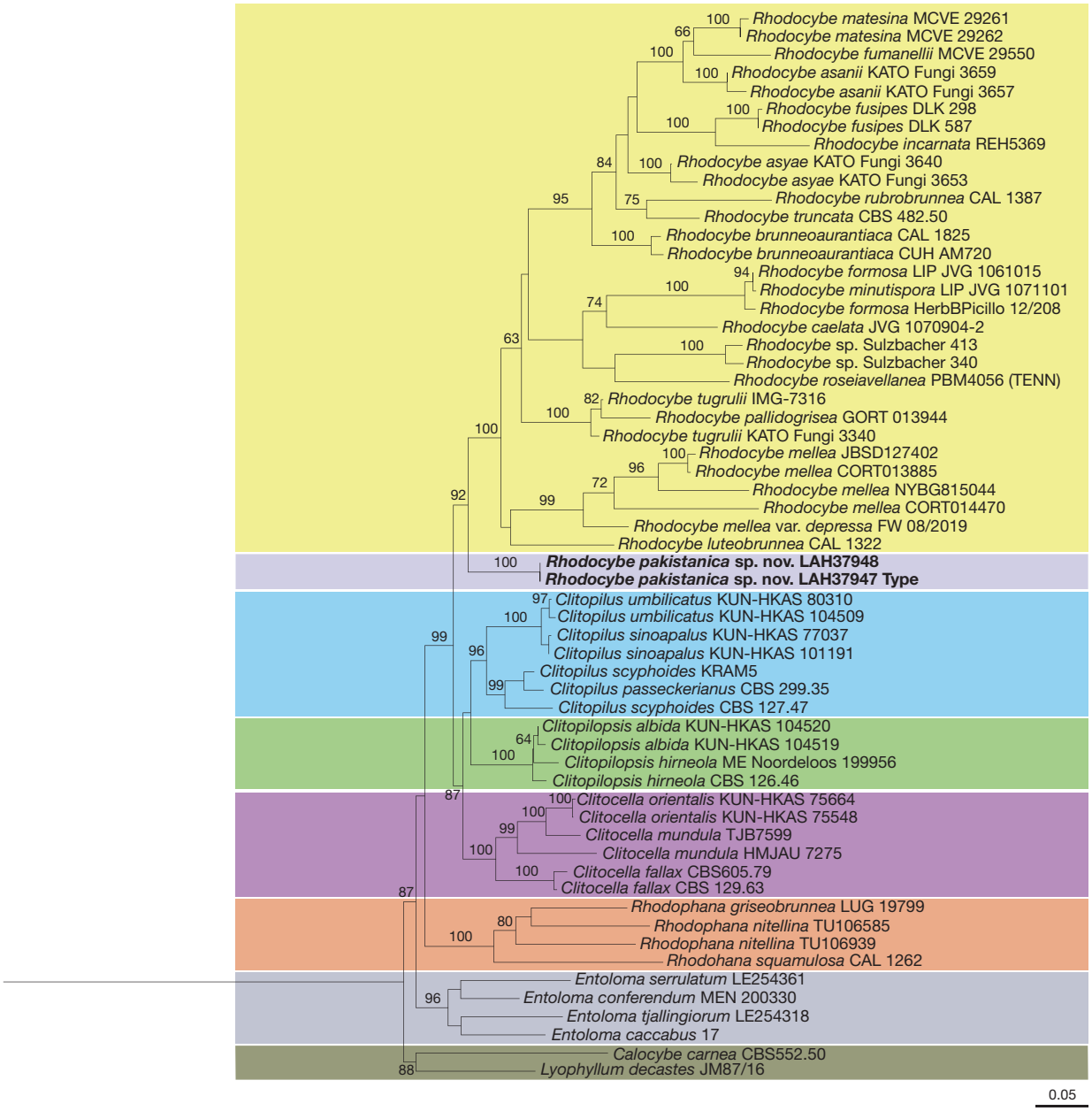

FIG. 2. — Maximum likelihood phylogenetic tree of sequences of Entolomataceae Kotl. & Pouzar, resulting from the analysis of combined nrITS-28S sequences; maximum likelihood bootstrap BT support values greater than 50% are written above the nodes; new species *Rhodocybe pakistanica* sp. nov. is indicated in **bold font**.

DISCUSSION

According to nrITS and combined ITS-28S phylogeny, *Rhodocybe pakistanica* sp. nov. forms a basal clade in *Rhodocybe*. The phylogenetic evolutionary tree supports *R. pakistanica* sp. nov. as separate from other *Rhodocybe* spp. for which ITS and 28S data exist.

Morphologically, *Rhodocybe mellea* T.J.Baroni & Ovrebo, a species originally described from Costa Rica, looks similar to *R. pakistanica* sp. nov. But it is distinct due to its wider pileus (19-42 mm), longer stipe (25-65 mm), subglobose or obovoid basidiospores, non-cyanophilic basidia, longer cheilo- and pleurocystidia (45-60 mm), and presence of caulocystidia (Ovrebo & Baroni 1988). *Rhodocybe mellea* var. *depressa* M.D.Xavier, Silva-Filho & Wartchow described from Brazil is mainly different due to its yellowish orange pileus, and cream-orange stipe which becomes enlarged towards the base, fleshy context, globose or subglobose basidiospores, and the presence of sclerobasidia (Xavier *et al.* 2022).

Rhodocybe pakistanica sp. nov. may be confused with *R. formosa* Vila, Contu, F.Caball. & A.Ortega, originally described from Italy. Still, the latter is different due to dark grey, beige-brown, ochre, and larger pileus (25-55 mm) covered with white pruina, grey-beige or pinkish lamellae,

FIG. 3. — Basidiomata of *Rhodocybe pakistanica* sp. nov.: **A**-**C**, LAH37948; **D**-**F**, LAH37947. Scale bars: 15 mm.

pale, dark grey, beige-brown, or ochre and larger stipe (25-60 × 4-10 mm) which is sometimes slightly off-center or flexuose, ellipsoid and pyriform basidiospores with suprahilar depression (Vizzini *et al.* 2016).

Rhodocybe brunneoaurantiaca A.K.Dutta, G.Gates & K.Acharya, described from India, can be distinguished by its smooth, shiny, and infundibuliform pileus with striate or rimose margins, brownish orange or light brown lamellae, larger stipe (24-37 × 5-7 mm) with smooth surface, non-cyanophilic basidia, flexuous cheilocystidia, and incrusted hyphae of stipitipellis (Dutta *et al.* 2021).

Rhodocybe tasmanica T.J.Baroni & G.M.Gates is different by its 7-11 mm broad pileus, lamellae with one tier lamellulae, shorter stipe (20-22 mm), amygdaliform or fusiform, and

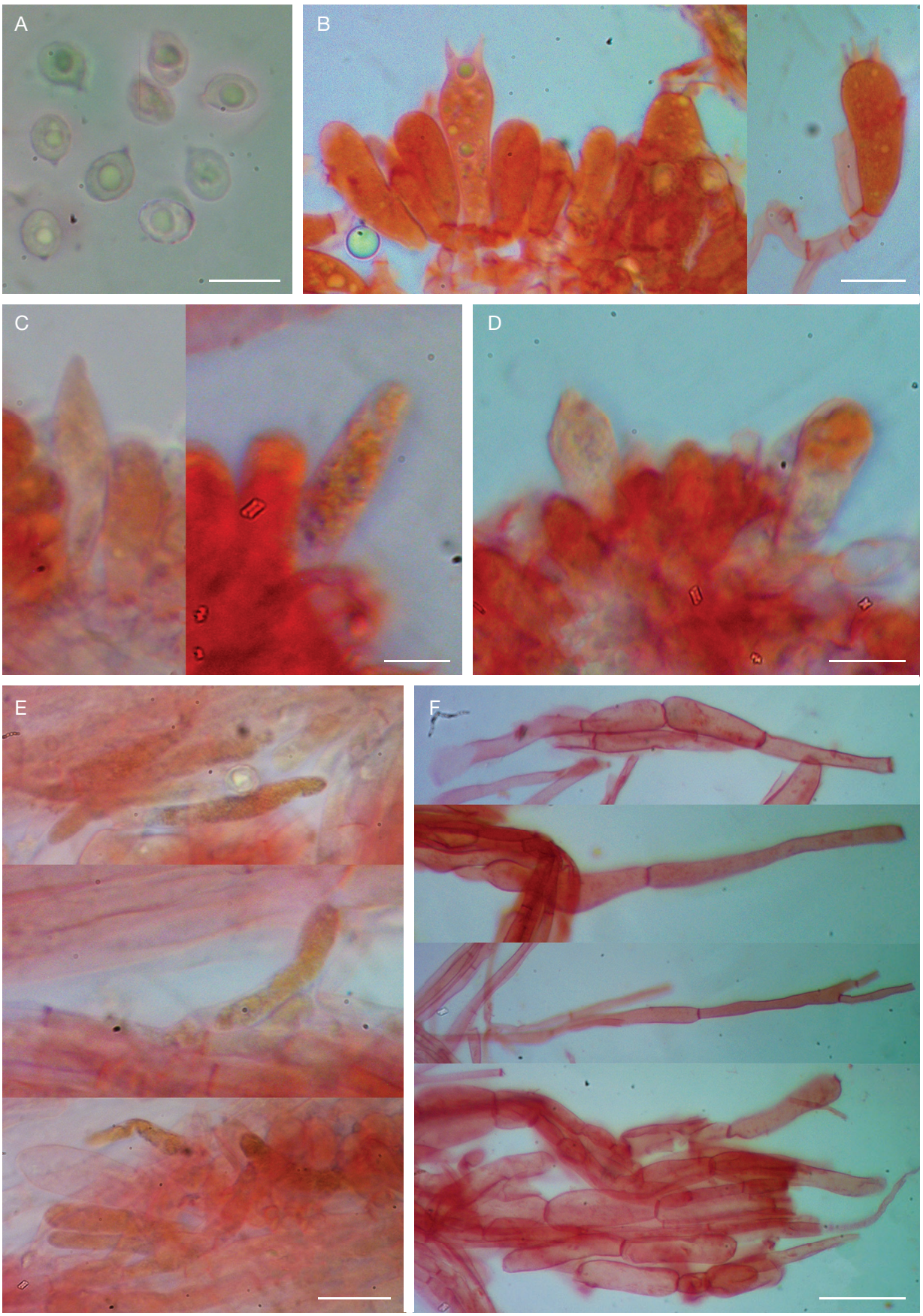

Fig. 4. — Microscopic characters of *Rhodocybe pakistanica* sp. nov. (LAH37947): **A**, basidiospores; **B**, basidia; **C**, **D**, cheilo- pleurocystidia; **E**, gloeoplerous hyphae in hymenial and pileal trama; **F**, stipitipellis. Scale bars: A, 8 µm; B, C, 10 µm; D, 16 µm; E, 20 µm; F, 25 µm.

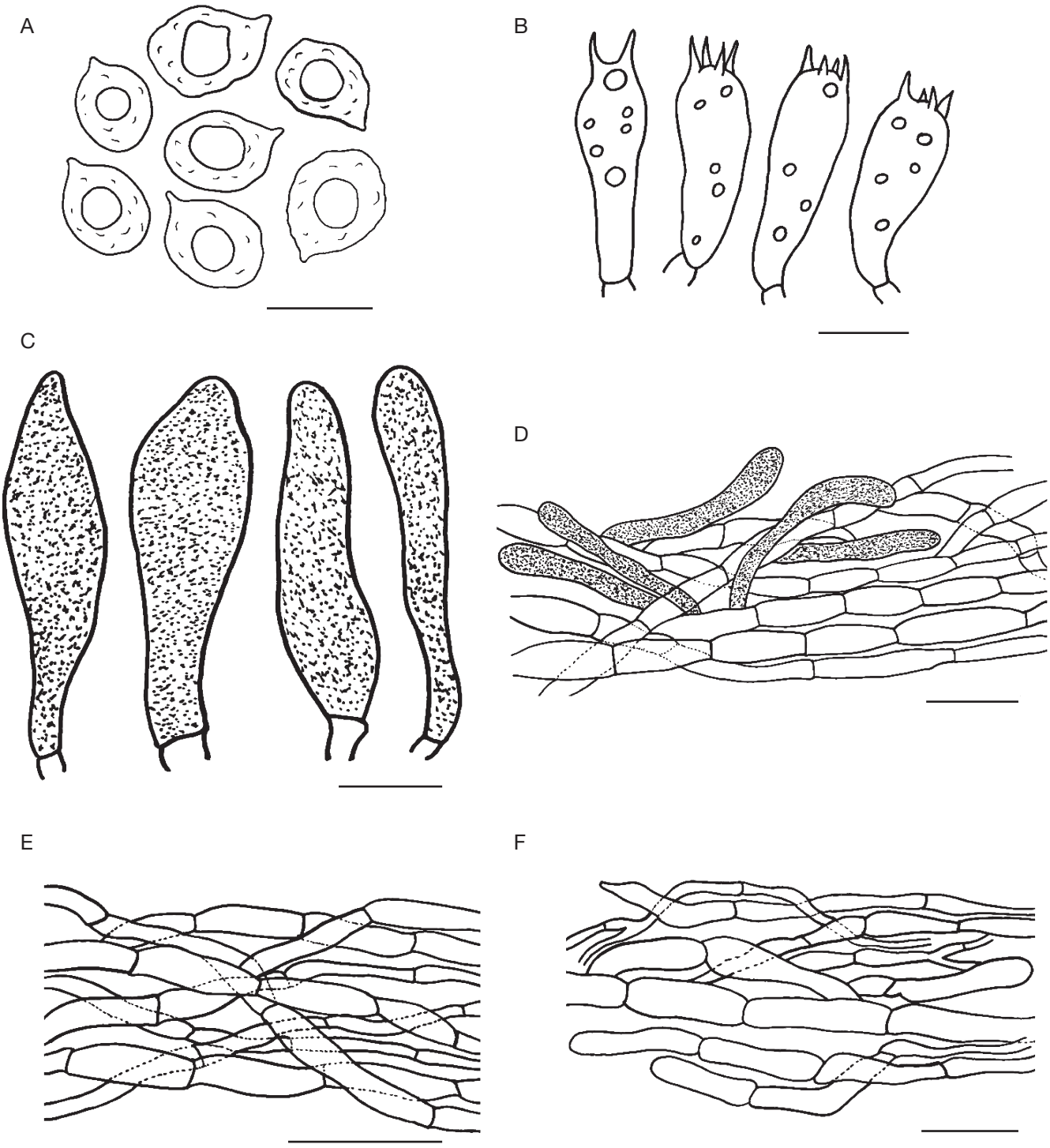

FIG. 5. — Line drawings of *Rhodocybe pakistanica* sp. nov. (LAH37947): **A**, basidiospores; **B**, basidia; **C**, cheilo- pleurocystidia; **D**, gloeoplerous hyphae in hymenial and pileal trama; **E**, pileipellis; **F**, stipitipellis. Scale bars: A, 6 µm; B, C, 10 µm; D, 20 µm; E, 12 µm; F, 25 µm.

larger basidiospores (7-12.5 × 4-5.6 µm), and longer basidia (25-36 µm) (Baroni & Gates 2006).

The characters that differentiate *Rhodocybe lateritia* T.J.Baroni & G.M.Gates include 21-85 mm broad pileus, pale pinkish lamellae, 5-20 mm wide and off-white stipe, and elliptical or subamygdaliform basidiospores (5.6-11 × 4.5-7.2 µm) (Baroni & Gates 2006).

Rhodocybe asyae Sesli & Vizzini can be distinguished by its whitish lamellae, stipe base with white-tomentose bulb, white to gray-beige context, wider hyphae of pileipellis (3-5 µm) and stipitipellis (7-16 µm) (Sesli & Vizzini 2017).

Rhodocybe brunnescens T.J.Baroni & E.Horak differs in having a slightly smaller (5-20 mm) pileus, grayish context, elongate or subamygdaliform basidiospores (6.5-9.5 × 4.5-5.5 µm), fusoid

or ventricose pseudocystidia (30-56 × 4-8 µm) and presence of caulocystidia (28-57 × 5.5-12 µm) (Baroni & Horak 1994).

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