

New species and record of Entolomatoid fungi (Entolomataceae, Agaricales) from Pakistan

Abdul Nasir Khalid

University of the Punjab Quaid-i-Azam Campus: University of the Punjab

Fauzia Razzaq

fauziarazzaq786@gmail.com

University of the Punjab Quaid-i-Azam Campus: University of the Punjab

Arooj Naseer

University of the Punjab Quaid-i-Azam Campus: University of the Punjab

Sobia Kanwal

University of the Punjab Quaid-i-Azam Campus: University of the Punjab

Tayyaba Qasim

University of the Punjab Quaid-i-Azam Campus: University of the Punjab

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Abstract

Taxonomic investigations on the family *Entolomataceae* have been conducted in Pakistan through surveys from 2018 to 2022. By combining morphological characteristics and molecular phylogenetic analyses using ITS sequences, three new species *Entoloma khanuspurensis*, *E. pakistanicum*, and *Rhodophana margallensis*, and one previously unrecorded species *E. albotomentosum* are introduced from Pakistan. This study marks the first report of the genus *Rhodophana* in Pakistan.

Introduction

The family *Entolomataceae* Kolt. & Pouzar is one of the most species-rich families within *Agaricales* and comprised more than 2, 200 species worldwide (Kirk 2019). Members of this family are cosmopolitan, ranging from arctic to tropical habitats and most species have been reported from Europe (Pegler 1977; Largent 1994; Noordeloos 2004; Gates and Noordeloos 2007; Noordeloos and Hausknecht 2007; Co-David et al. 2009; Castellano et al. 2011; Karstedt and Capelari 2013; Smith et al. 2015; Sulzbacher et al. 2020; Elliott et al. 2020; Dima et al. 2021; Reschke et al. 2022). Many species are saprotrophic on soil and litter, while some are ectomycorrhizal and found to be associated with conifers and angiosperms (Co-David et al. 2009; Kondo et al. 2017). The family is characterized by pinkish-brown spore print, and angular spores in both face and polar views (Noordeloos et al. 2022; Seidmohammadi et al. 2021). The *Entolomataceae* comprises two major clades, one is *Entoloma* clade, and the other *Rhodocybe-Clitopilus* clade (Co-David et al. 2009; Baroni et al. 2011; Baroni and Matheny 2011; Kluting et al. 2014). The *Rhodocybe-Clitopilus* clade has taxa approximately 300 globally, while the *Entoloma* clade contains 2000 species worldwide (Noordeloos et al. 2018). The phylogenetic analysis of *Entolomataceae* was conducted by Co-David et al. 2009; Baroni and Matheny 2011; Kluting et al. 2014. The studies on the clade *Rhodocybe-Clitopilus* by Kluting et al. (2014), recognized five distinct genera based on morphological characters: *Rhodocybe* Maire (1937:113), *Rhodophana* Kühner (1971:23), *Clitopilopsis* Maire (1973:113), *Clitopilus* (Fr. ex Rabenh.) P. Kumm. (1871:23) and a newly described genus *Clitocella* Kluting, T.J. Baroni & Bergemann (2014:1132) which is a sister group to *Clitopilus* and *Rhodocybe*. The generic concepts given by Kluting et al. (2014) have been followed.

The species-rich genus *Entoloma* (Fr.) P. Kumm. (1871:143) is characterized by its pinkish-brown spore print and with almost polyhedroid, faceted basidiospores, which are angled in outline (Noordeloos 1980; Noordeloos 1981, 1984; Singer 1986; Gates and Noordeloos 2007; Noordeloos and Hausknecht 2007; Noordeloos and Morozova 2010; Noordeloos et al. 2021; Reschke et al. 2022). This genus is divided into several sub-genera; *Claudopus*, *Cyanula*, *Entoloma*, *Inocephalus*, *Leptonia*, *Nolanea*, *Pouzarella*, *Richoniella* and *Trichopilus* (Largent 1994; Noordeloos and Gates 2012). Only 12 species of the genus *Entoloma* have previously been reported from Pakistan (Ahmad 1962; Ahmad et al. 1997; Sultana et al. 2011; Haelewaters et al. 2020; Khalid 2022; Izhar et al. 2022, 2023). Here, we presented two new species of *Entoloma* subg. *Entoloma* and one new record viz., *E. albotomentosum* subg. *Claudopus* from Pakistan. These species are described on the basis of micro-morphological and phylogenetic analyses using nrITS and LSU regions.

Rhodophana Kühner is a genus in the family *Entolomataceae* that was defined by Kluting et al. (2014). This genus was first described by Kühner with *R. nitellina* (Fr.) Papetti, as the type species (Kühner 1947). Previously, Kühner redescribed it as *Rhodocybe* subgenus (Kühner and Lamoure 1971). Based on phylogenetic analysis, Kluting et al. (2014) revealed that it belonged to an independent branch, so it was resurrected as a new genus. Current research predicted that there will be many new combinations of the genus *Rhodophana* (Buyck et al. 2021). This genus is characterized by collybioid basidiocarps, adnexed to adnate lamellae and undulate-pustulate ornamentation and with more or fewer clamp connections (Raj et al. 2015; Xu et al. 2023). Since there are 16 valid records in Index Fungorum, distributed in Asia, the Canary Islands of Africa, and Europe (Vizzini et al. 2011a, 2012; Raj et al. 2015; Yang and Fan 2020; Buyck et al. 2021; Xu et al. 2023). Here, we present *R. pakistanensis* as new species and this is the first report of the genus from Pakistan. By conducting field surveys and collecting entolomatoid fungi from various regions of Pakistan, we intend to document the occurrence of these species and identify their taxonomic diversity.

Materials and Methods

Samples collection

All the specimens were collected from different areas of Pakistan including Khanuspur, Lahore, Margalla Hills, and Parachinar during macrofungal surveys from 2017–2021. These collected samples were air-dried with a dehydrator at 45°C and deposited in the Lahore Herbarium (LAH) of the Institute of Botany, University of the Punjab, Lahore, Pakistan.

Macro- and micro-morphological analyses

The macromorphological descriptions were based on the specimen photographs captured during sampling and filed notes. Samples images were taken in natural light using a Nikon D70 camera. The color chart described by Munsell (1975) was used. The micromorphology of the samples was studied using a CXRII, Lambomed, Labo America Inc., Fremont, CA, USA compound microscope at different magnifications. All measurements were observed under a 40X and 100X oil immersion. Free-hand sections from dried specimens were made and mounted in 5% KOH, Congo red, and Melzer's reagents for observations. At least 60 measurements of each micro-characteristic (basidia, cystidia, basidiospores, elements of pileipellis, and stipitipellis) were taken. Dimensions were represented as (a–) b – c (– d); here 'a' and 'd' were meant as extreme values in parentheses and 'b – c' covered nearly 90% of measured values. Factor Q is the ratio of basidiospore length to width and Q_{av} is the average of factor Q.

Molecular procedures and sequencing

Genomic DNA was extracted from dried specimens following the methodology described by Bruns (1995). For PCR amplification, primer pair ITS1 and ITS4 (White et al. 1990) were used for the nrITS region. The PCR products were observed on a 1% agarose gel and then sent to China (TsingK) for sequencing.

Phylogenetic analyses

The sequences generated during this study, and the reference taxa sequences showing identity > 90% were retrieved from GenBank, along with sequences from recently published papers (Vizzini et al. 2016; Brandrud et al. 2018; Reschke et al. 2022). *Lyophyllum leucophaeatum* (P. Karst.) P. Karst. was selected as an outgroup for nrITS-based phylogenetic analysis (Vizzini et al. 2016). The ITS dataset was aligned using an online tool MUSCLE by EMBL-EBI (Edgar 2004) and then manually adjusted in BioEdit 7.0.5.3 (Hall 1999). Maximum Likelihood analysis of the nrITS dataset was carried out using RAxML-HPC2 v 8.1.11 (Stamatakis 2014) via the CIPRES portal (Miller et al. 2010) with 1000 BS replicates. The final phylogram was displayed in FigTree v 1.4.3 (Rambaut 2014) and exported to Adobe Illustrator.

Results

Phylogenetic results

The ITS data matrix contained total of 76 sequences including the outgroup. The final aligned dataset comprised of total 1089 positions, out of which 427 were conserved characters, 639 as variables, and 509 as parsimony-informative. In the phylogenetic analysis based on nrITS region, two *Entoloma* subg. *Entoloma* and one *Rhodophana* Pakistani species sequence made separate branches with closely related species sequences. While one *Entoloma* species viz., *E. albotomentosum* subg. *Claudopus* clustered with the sequences of the same species which were retrieved from GenBank with strong bootstrap support (Fig. 1).

Taxonomy

Entoloma khanspurensis S. Kanwal, & Khalid sp. nov. Figure 3

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Etymology: –The specific epithet '*khanspurensis*' refers to the type locality Khanspur, a village near Ayubia National Park, KP.

Diagnosis

Pileus brownish dull red, bilobed and bilabiate pileus, adnate lamellae, smaller (7.4 – 11.9 × 6.3 – 9.2 μm) basidiospores with 3 – 6 angles, and presence of cylindrical cheilocystidia.

Type: -PAKISTAN. Kyber Pakhtunkhwa: Abbottabad District, Khanspur, on moist rich soil, 73°–22.8 to 73°–27.1 E and 34°–3°8 N, 2575 m, August 2019, Abdul Nasir Khalid, (AY–31).

Basidiocarp 4.7 cm in height, annual, terrestrial, fleshy, solitary, medium-sized. **Pileus** 3.0 cm in diameter, convex, bilobed and bilabiate, margins wavy, slightly scrobiculate, dull reddish brown (5YR5/3), moist,

and uneven surface. **Lamellae** adnate, unequal, incurved, light yellowish gray (10YR8/2), closed to subdistant, narrow and average in thickness, eroded margins, gills concolorous with a stipe, lamellulae almost present in 2 tiers. **Stipe** 4.4 cm in length, centrally attached, equal, cylinder, light yellowish gray (10YR 8/2), firmly attached to the pileus. **Odor** and **Taste** not recorded.

Basidiospores (7.4-) 8.3 – 10.1 (- 11.9) × (6.3-) 6.6 – 8.7 (- 9.2) μm, avl × avv = 9.4 × 7.1 μm, Q = 1.1–1.2 μm, avQ = 1.3 μm, with 3–6 angled, transparent in 5% KOH. **Basidia** (33.2-) 35.1 – 43.2 (- 45.1) × (11.4-) 12.4–14.2 (- 14.8) μm, avl × avw = 39.9 × 13.5 μm, clavate, bisporic to tetra-sporic, 2–4 sterigmata slightly pointed, hyaline in 5% KOH. **Cheilocystidia** (17.5-) 24.0–28.3 (- 40.1) × (4.7-) 7.2–9.1 (- 9.2) μm, avl × av = 26.3 × 8.07 μm, cylindrical, hyaline in 5% KOH, thin-walled. **Pileipellis** (4.7-) 5.5 – 7.8 (- 8.0) μm, avw = 6.5 μm, densely packed, subregular and parallel hyphae, hyaline, cylindrical, septate, interwoven, thin-walled. **Stiptipellis** (3.9-) 4.2 – 8.2 (- 10.1) μm, avw = 6.4 μm, intermixed, hyaline, septate, and thin-walled. Clamp connections are present in all tissues.

Ecology

Solitary on moist rich soil in Khanspur.

Material Examined

PAKISTAN, Khyber Pakhtunkhwa Province, Abbottabad District, Khanspur, 2575 m a.s.l, August 2019, Abdul Nasir Khalid, (AY-31).

Entoloma pakistanicum Khalid, Naseer, A., sp. nov. Figure 4

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Etymology

The specific epithet '*pakistanicum*' refers to the country Pakistan, its type locality.

Diagnosis

Pileus brown, umbilicate, convex, incurved margins. Lamellae creamy-white with serrate margins. Stipe fibrillose and creamy white with a yellow tinge at the lower portion. Basidiospores (11.7 × 9.2 μm), subspherical.

Type: - PAKISTAN, Khyber Pakhtunkhwa Province, Kurram District, Parachinar, 30 July 2019, Arooj Naseer & Abdul Nasir Khalid, ANK252 (GenBank accessions ITS MW218151; LSU MW142504).

Description: **Basidiocarp** medium-sized. **Pileus** 3.2–4 cm in diameter, convex, umbilicate, light brown (7.5Y 7/2) in the center and slightly darker brown (5Y 7/2) towards margins, becoming dark brown (5Y 7/2) when mature, smooth, incurved margins. **Lamellae** adnexed, creamy white (1.3BG 5/0.6), serrate

margins, broad, close, lamellulae regular, present in one-tier. **Stipe** 5–5.7 cm in length, 0.9 cm width at apex, 1.2 in the center, and 0.7 cm width at base, compressed, slender, fibrillose, creamy white (1.3BG 5/0.6), with a yellow tinge in the lower portion, equal or broadening towards the base, with a yellow tinge, dry, solid, central. **Odor** aromatic resembling fruit and **Taste** not recorded.

Basidiospores (9.9–) 10.1–13.1 (–13.9) × (7.1–) 7.9–10.8 (–11.1) μm, avl × avw = 11.7 × 9.2 μm, Q = 1–1.7, Qav = 1.3, subspherical, 4–6 angled, smooth, inamyloid, hyaline in 5% KOH, thick-walled. **Basidia** 33.5–57.4 × 11.5–17.4 μm, avl × avw = 44.8 × 14.6 μm, clavate to broadly clavate, hyaline in 5% KOH, thin-walled, 2–4 spored. **Cheilocystidia** 21.6–29 × 8–9.9 μm, avl × avw = 26.2 × 9.1 μm, narrowly clavate to utriform, hyaline in 5% KOH, thin-walled. **Pileipellis** 3.9–14.2 μm, avw = 8.1 μm, septate, branched, hyaline to light brown in 5% KOH, thick-walled, some hyphae slightly encrusted, terminal cells cylindrical, long, tapering. **Stiptipellis** 4.2–16.7 μm, avw = 9.9 μm, septate, branched, hyaline in 5% KOH, thick-walled, terminal cells long, clavate or cylindrical. Terminal cells of stipe 106.1–133 × 20.4–27.1 μm, avl × avw = 118.4 × 21.8 μm, clavate to oblong, hyaline in 5% KOH, thick-walled. Clamps are abundant in all structures.

Ecology

Growing on loamy soil under *Quercus baloot* in a moist temperate oak forest.

Material examined

PAKISTAN, Khyber Pakhtunkhwa Province, Kurram District, Parachinar, July 2019, Arooj Naseer & Abdul Nasir Khalid, ANK-252, ANK-360.

Entoloma albotomentosum Noordel. & Hauskn., *Z. Mykol.* 55(1): 32 (1989) Fig. 5

Description: **Basidiocarps** small in size. **Pileus** 0.5–1.7 cm in diameter, hemispherical becoming convex with a central depression, context thin with very less flesh, light pinkish brown (10R 8/4), surface smooth, powdery or tomentose with minute hairs, light pinkish brown, margins equal, dentate, slightly appendiculate, furcated in 4 or 5 portions upon maturity. **Lamellae** adnate, non-decurrent, thin, less wide, distant, light pinkish brown (10R 8/3) in color, margins entire, lamellulae present alternating with lamellae. **Stipe** 1–2.6 × 0.1–0.3 cm, central, equal to sub-equal cylindrical, broad upwards, tapering towards base, context thin, pinkish white (10R 8/2), surface smooth, powdery, base slightly swollen. **Annulus** and **Volva** absent.

Basidiospores (9.2–) 9.5–11.1 (–11.5) × (7–) 7.3–8.6 (–8.9) μm, avl × avw = 10.2 × 7.8 μm, Q = 1.27–1.32, avQ = 1.29, angular with 7–8 angles, not sharp, thick walled, non-dextrinoid, apiculate, without germ-pore, large central guttule present along with two or three small guttules. **Basidia** (27.8–) 28.3–34.6 (–35.5) × (9.4–) 9.8–13.3 (–14.5) μm, avl × avw = 31.6 × 14.5 μm, sub-clavate, cylindrical, sub-cylindrical, smooth-walled, sometimes walls rugulose, mostly 2 spored, rarely 4 spored, no short cells or clamps present at base, internal contents not visible. **Cheilocystidia** and **Pleurocystidia** absent. **Pileipellis** a cutis made up of thin-walled hyaline hyphae, 4.1–19 μm in diameter, avw = 9.2 μm, smooth-walled, septate,

branched, terminal ends equal. **Stipitipellis** a cutis made up of thin-walled hyaline hyphae, 2–13 µm in diameter, avw = 7.1 µm, parallel to irregular in arrangement, smooth, no internal contents found, septate, branched, no clamp connections present, terminal ends equal.

Ecology

Saprotrophic, omphaloid, scattered or present in groups of 2 or 3 on dead decaying organic matter.

Material Examined: PAKISTAN, PUNJAB, Lahore, University of the Punjab Lahore, 217 m a.s.l., 1st August 2017, Tayyaba Qasim B6 (LAH36551), GenBank: XXX.

Rhodophana margallensis F. Razzaq, Khalid, sp. nov. Figure 6

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Etymology: –The specific epithet '*margallensis*' refers to the type locality, Margalla Hills Pakistan.

Diagnosis

Pileus dull yellow-orange with a reddish-brown center, sinuate lamellae develop collarium around stipe, broadly ellipsoidal large (6.20–9.63 × 4.72–7.27 µm) basidiospores, and presence of hymenial cystidia.

Type

PAKISTAN. Islamabad, Margalla Hills National Park, found in small groups on soil litter and humus in Margalla Hills, temperate, sub-humid, and monsoon climate, at 1604 m a.s.l., August 2020, Shazia Ashraf, MH34.

Description: **Basidiocarps** 3–6 cm in height, small to medium-sized, solitary to gregarious, dry and dull. **Pileus** 2–4 cm in diam., convex to hemispherical with central depression at maturity, broadly parabolic when young, deeply indented, very dark reddish brown (5YR2/4) at the center, and appressed scales, dull yellow-orange (10YR6/4) to dull orange (7.5YR6/4) elsewhere, hard, surface dull and dry, squarrose, margins eroded. **Lamellae** sinuate, collarium around stipe, close to crowded, broad, bright brown (10YR6/6) to light orange (10YR8/4), arid, unequal, margins eroded, with 3–4 lamellulae among two lamellae. **Stipe** 2–4 × 0.5–1 cm, central, clavate, reddish-yellow (7.5YR6/6) to light yellow-orange (10YR8/3–8/4), fibrillose, rigid, context white, with white basal mycelial pad. **Annulus** and **Volva** absent. **Taste** and **Odor** not recorded.

Basidiospores (6.20–) 6.31–9.41 (–9.63) × (4.72–) 5.09–7.25 (–7.27) µm, avl×avw = 7.93×6.08 µm, Q = 1.07–1.55 µm, Qav = 1.30 µm, broadly ellipsoidal, apiculate, monoguttulate, uneven, undulate-pustulate all over the surface, thin-walled, hyaline with greenish guttulate in 5% KOH. **Basidia** (25.37–) 27.98–34.24 (–34.99) × (9.14–) 9.941–10.18 (10.61) µm, avl×avw = 29.85×9.73 µm, clavate, broader at upper side,

with 2–4 sterigmata, guttulate, thick-walled, hyaline to dull yellow in 5% KOH. **Cheilocystidia** (22.80–) 22.95–25.43 (–32.0) × (6.03–) 6.63–7.72 (–9.34), $avl \times avw = 25.79 \times 7.43 \mu\text{m}$, utriform to narrowly utriform, obovoid, thin-walled, hyaline in 5% KOH. **Pileipellis** euhymeniderm, composed of both narrow and inflated hyphae, 4.66–18.58 μm in diam., septate, regular, smooth, thin-walled, hyaline in 5% KOH. **Stiptipellis** a cutis, hyphae 5.30–8.05 μm in diam., $avw = 7.16 \mu\text{m}$, parallel in arrangement, cylindrical, septate, smooth, thin-

walled, clamp-connection present, yellowish with greenish walls in 5% KOH, caulocystidia absent.

Ecology

Growing in small groups on litter and humus.

Specimen examined

PAKISTAN. Islamabad, Margalla Hills National Park, found in small groups on soil litter and humus in Margalla Hills, 1604 m a.s.l., August 2020, Shazia Ashraf MH34, MH334.

Discussion

In the present study, we described five species of the family *Entolomataceae*, among them four belong to the genus *Entoloma* and one to *Rhodophana* from various sites in Pakistan. The identification has been based on their morphological features and rDNA sequence data.

Entoloma khanspurensis sp. nov. in subgenus *Entoloma*, belongs to the *Sinuatum* clade and is characterized by dull reddish brown bilobed and bilabiate pileus, adnate lamellae with 2-tiers, relatively smaller (7.4 – 11.9 × 6.3 – 9.2 μm) basidiospores with 3 – 6 angled, and presence of cylindrical cheilocystida. The phylogenetically close species, *E. bisporigerum* (P.D. Orton) Noordel., can be differentiated by its plain gray-brown to pale-brown pileus, large-sized (11.5 – 13.5 × 7.5 – 9.5 μm) basidiospores with 6 – 9 angled, and absence of cystidia (He et al. 2016).

Species in subgenus *Entoloma* within *Rhodopodium* clade are characterized by hygrophanous, smooth, or rarely slightly fibrillose pileus and fibrillose (usually white, grey, or brown) stipe. The spores are usually subisodiametrical or broadly heterodiametrical with distinct angles, and clamps are present. *Entoloma pakistanicum* sp. nov. is characterized by brown, umbilicate, convex pileus, creamy white gills, fibrillose and creamy white stipe, and subisodiametrical, larger basidiospores (11.7 × 9.2 μm). Phylogenetically close specimen *E. rhodopodium* Berk. & Broome can be differentiated on the basis of the olive-gray color pileus, emarginate lamellae, and small-sized (7.3 × 6.6 μm) globose basidiospores (Ouabbou et al. 2017).. Molecular phylogenetic analyses based on ITS sequences also support *Entoloma pakistanicum* as a distinct species with strong bootstrap support.

Entoloma albotomentosum Noordel. & Hauskn., which belongs to the *Entoloma* subgenus *Claudopus* is recorded for the first time from Pakistan. This species was originally described from Austria by

Noordeloos and Hausknecht (1989) characterized by white pleurotoid basidiocarps, an excentric stipe. Anatomically, 10–11 × 6.5–8 µm basidiospores, a cutis pileipellis made up of 4–12 µm wide hyphae and lack of clamp connections. Since then, it was found in several other countries viz. Netherlands, Liechtenstein, Germany, Denmark, England, Scotland, Norway, Poland, Slovakia, and India (Krieglsteiner 2003; Legon et al. 2005; Arnolds and Veerkamp 2008; Noordeloos 2008; Halma 2011; Jančovičová and Adamčík 2014; Acharya et al. 2017). Our collection of *E. albotomentosum* mostly agrees with the type specimen except for its light pinkish-brown pileus, it might be due to different climatic conditions.

Rhodophana margallensis sp. nov. is characterized by dull yellow orange to dull orange pileus except in the center, sinuate lamellae develop collarium around stipe and clavate stipe. Moreover, it has broadly ellipsoidal (6.20–9.63 × 4.72–7.27 µm) basidiospores and the presence of hymenial cystidia and euhypheniderm pileipellis. Phylogenetically, this new taxon clustered with *R. squamulosa* K. P. D. Latha & Manim., *R. griseobrunnea* E. Musumeci, and *R. nitellina* (Fr.) Papetti. The phylogenetically close relative *R. squamulose* can be different from *R. margallensis* by initially convex, becoming infundibuliform pileus with central depression when mature, greyish red pileus in color except for the center, adnexed lamellae, terete to equal or slightly tapering towards base stipe, lacrymoid or subglobose small-sized basidiospores [(5.5–) 6–8 × 4–5.5 µm], absence of hymenial cystidia and disrupted cutis pileipellis with ascending trichodermal patches (Raj et al. 2015). Phylogenetically close relative *R. griseobrunnea* can be different from the newly described taxon by its brownish-colored basidiocarps with strong farinaceous odor, and large-sized basidiospores (7–11 (13) µm in length) (Musumeci 2020). Another phylogenetically close species *R. nitellina* can be distinguished from *R. margallensis* by orange-brown to red-brown glabrous pileus, pale brownish cream lamellae, ellipsoidal basidiospores, absence of cystidia, and a cutis pileipellis (Noordeloos 1988).

Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

Data availability

All Specimens are deposited in Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan, descriptions to MycoBank and sequences have been deposited to GenBank. Alignment file can be obtained from the first author.

Contributions

Abdul Nasir Khalid and Arooj Naseer collected field samples. Fauzia Razzaq, Arooj Naseer, Sobia Kanwal and Tyabba Qasim contributed the morphological and molecular analyses under the guidance of Abdul Nasir Khalid. Arooj Naseer contributed to the study conception and design. The first draft of the manuscript was prepared by Fauzia Razzaq and carefully revised by all authors.

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Tables

Table 1

A list of species, origins, locality, voucher numbers, GenBank accession numbers, and references of taxa used for the nrITS-based phylogenetic analysis. Sequences generated in this current study are shown in bold.

Species	Locality	Voucher	GenBank accessions	References
Entoloma aurorae-borealis	Norway	O-F-254651 / JL 85 - 14	MH234486	Noordeloos et al. 2018
Entoloma aurorae-borealis	Norway	O-F-76218 / JL 67 - 16	MH234487	Noordeloos et al. 2018
Entoloma aurorae-borealis	Norway	O-F-76217 / JL118-16	MH234484	Noordeloos et al. 2018
Entoloma serpens	Norway	O-F-303802 / GB 4713	MH234491	Noordeloos et al. 2018
Entoloma serpens	Norway	O-F-253866 / EB 94/16	MH234488	Noordeloos et al. 2018
Entoloma serpens	Finland	KK410-09	LN850526	Kokkonen 2015
Entoloma bisporigerum	Norway	OW-E20-14	MZ868976	Reschke et al. 2022
Entoloma bisporigerum	Finland	KK 934/03	LN850535	Kokkonen 2015
Entoloma bisporigerum	Finland	KK 106/02	LN850534	Kokkonen 2015
Entoloma sp.	China	130822MFBPC312	MW554153	Unpublished
Entoloma sp.	China	110116MFBPC492	MW554247	Unpublished
Entoloma khanspurensis	Pakistan	————	————	This study
Entoloma khanspurensis	Pakistan	————	————	This study
Entoloma sp.	Netherlands	MEN2012125	MZ868977	Reschke et al. 2022
Entoloma murinum	USA	MUOB:355239	OK376736	Unpublished
Entoloma borgenii	Norway	O-F-76219 / JL 51 - 16	MH234482	Noordeloos et al. 2018
Entoloma borgenii	Norway	O-F-76220 / JL 63 - 16	MH234483	Noordeloos et al. 2018

Species	Locality	Voucher	GenBank accessions	References
Entoloma borgenii	Finland	KK 307/05	LN850519	Kokkonen 2015
Entoloma nidorosum	Greenland	JHP-380	LN850509	Kokkonen 2015
Entoloma nidorosum	Sweden	11356	OB998032	Kokkonen 2015
Entoloma nidorosum	Sweden	GG160815	MZ868997	Reschke et al. 2022
Entoloma politum	Finland	KK 111/02	LN850512	Kokkonen 2015
Entoloma politum	Finland	KK 289/09	LN850511	Kokkonen 2015
Entoloma rhodopolium	Japan	KUB104	LC088043	Kondo et al. 2017
Entoloma rhodopolium	Japan	KUB105	LC088044	Kondo et al. 2017
Entoloma rhodopolium	Japan	KUB106	LC088045	Kondo et al. 2017
Entoloma pakistanicum	Pakistan	———	———	This study
Entoloma pakistanicum	Pakistan	———	———	This study
Entoloma sp.	USA	MLS007	GQ397994	Unpublished
Entoloma sp.	USA	iNat97785436	OP681754	Unpublished
Entoloma spadiceum	USA	iNat97785436	OP104078	Unpublished
Entoloma cf. spadiceum	USA	CNH15	MF686497	Unpublished
Entoloma cf. spadiceum	USA	Mushroom Observer 468771	OM039442	Unpublished
Entoloma lividoalbum	France	GE12-025	MZ868957	Reschke et al. 2022
Entoloma bicolor	Canada	3850	KJ705162	Unpublished
Entoloma bicolor	USA	F27587	MZ314302	Unpublished

Species	Locality	Voucher	GenBank accessions	References
Entoloma majaloides	Italy	E. Campo 25. VIII.2009	LN850492	Kokkonen 2015
Entoloma majaloides	Finland	KK 159/07 & J. Vauras	LN850486	Kokkonen 2015
Entoloma neglectum	Finland	KK 158/05	LN850557	Kokkonen 2015
Entoloma cremeoalbum	Finland	O:300037	NR_152933	Kokkonen 2015
Entoloma neglectum	Finland	V. Liiv & K. Kalamees, J. Vauras 20020	LN850558	Kokkonen 2015
Entoloma cremeoalbum	Canada	MQ22-KEG001-HRL3422	OQ32188	Unpublished
Entoloma neglected	China	KUN-HKAS 115923	MZ855873	He and Yang, 2022
Entoloma sp.	China	HMLD1051	KC257439	Unpublished
Entoloma alpinum	China	SAAS 774	KJ658969	He et al. 2014
Entoloma cettoi	Italy	WU: 11500	NR_138020	Kokkonen 2015
Entoloma albotomentosum	Philippines	BP28	MZ725488	Unpublished
Entoloma albotomentosum	Pakistan	————	————	This study
Entoloma albotomentosum	India	CUH:AM253	KX904354	Acharya et al. 2017
Rhodophana margallensis	Pakistan	————	————	This study
Rhodophana margallensis	Pakistan	————	————	This study
Rhodophana squamulosa	India	CAL:1262	NR_155719	Raj et al. 2015
Rhodophana squamulosa	India	CAL:1262	KT180329	Raj et al. 2015
Rhodophana griseobrunnea	France	LUG 19799	MT580804	Musumeci 2020

Species	Locality	Voucher	GenBank accessions	References
<i>Rhodophana griseobrunnea</i>	France	LUG:19799	NR_173192	Musumeci 2020
<i>Rhodophana</i> cf. <i>nitellina</i>	Mexico	MushroomObserver.org/241518	MH058041	Unpublished
<i>Rhodophana nitellina</i>	USA	MF80542/NS1930	OM906885	Unpublished
<i>Clitopilus cystidiatus</i>	France	TO AV131	HM623130	Vizzini et al. 2011b
<i>Clitopilus cystidiatus</i>	Netherlands	E. Arnolds 03–27	KC885964	Morgado et al. 2016
<i>Clitopilus prunulus</i>	USA	TJB6838	DQ202272	Unpublished
<i>Clitopilus prunulus</i>	United Kingdom	CBS 227.93	FJ770408	Hartley et al. 2009
<i>Rhodocybe fallax</i>	Switzerland	CBS605.79	AF357018	Hofstetter et al. 2002
<i>Rhodocybe fallax</i>	Switzerland	CBS129.63	AF357017	Hofstetter et al. 2002
<i>Clitopilus amarus</i>	Netherlands	A. d. Haan 98031	KC885963	Morgado et al. 2016
<i>Rhodocybe formosa</i>	Italy	Herb. B. Picillo 12/198	KU862857	Vizzini et al. 2016
<i>Rhodocybe minutispora</i>	Spain	LIP JVG 1071101	KU862860	Vizzini et al. 2016
<i>Lyophyllum leucophaeatum</i>	Switzerland	Hae251.97	AF357032	Hofstetter et al. 2002

Figures

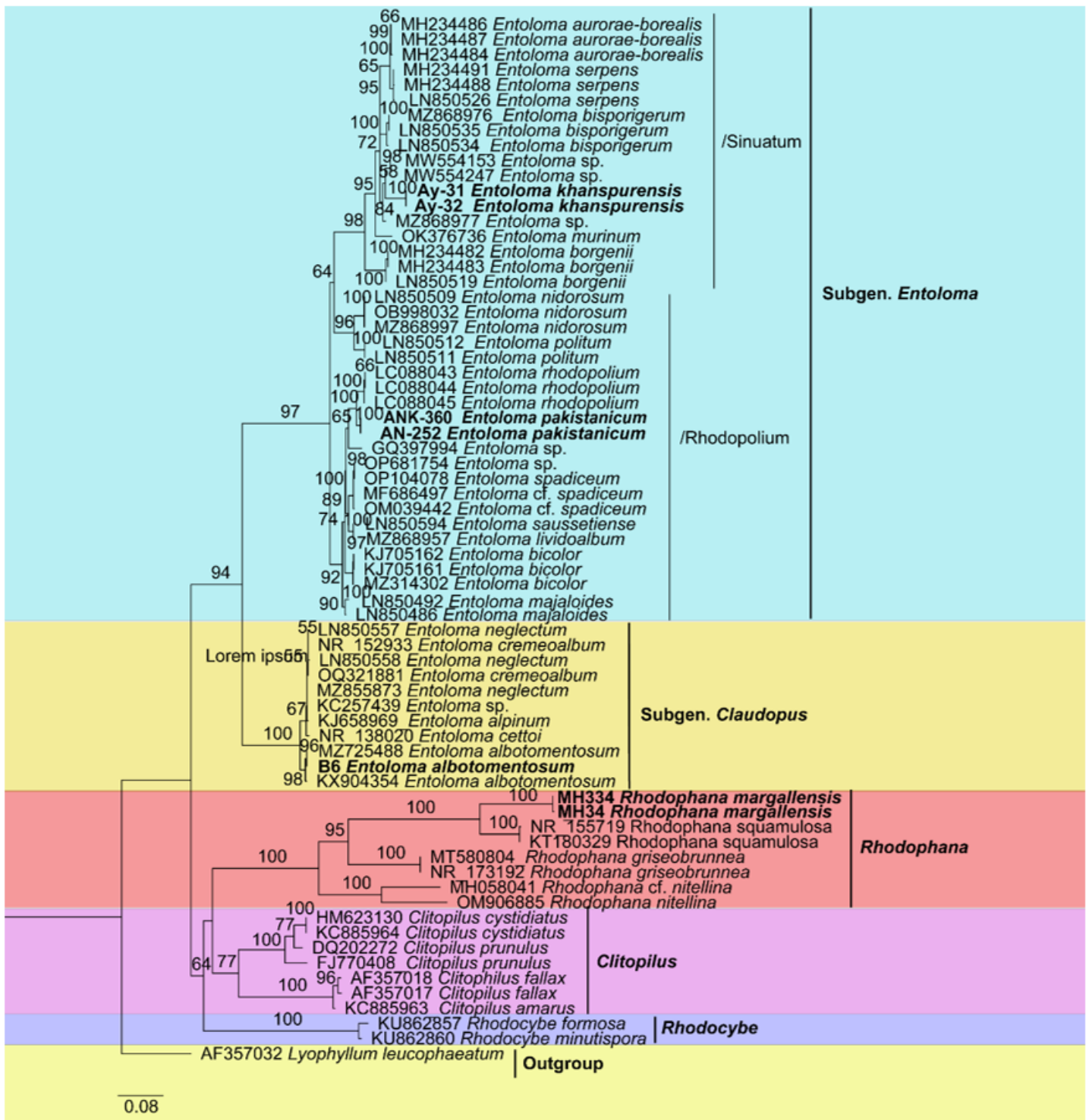


Figure 1

Maximum likelihood phylogenetic analysis based on the ITS sequences of *Entolomataceae*, with *Lyophyllum leucophaeatum* as outgroup taxa. MLB values Line \geq are shown on the branches and newly generated sequences are represented in bold.



Figure 2

A-L: Basidiocarps of collected specimens in their natural habitat. A-C. *Entoloma albotomentosum*, D-E. *E. khanspurenis*, E-I. *E. pakistanicum*, J-L. *Rhodophana margallensis*.

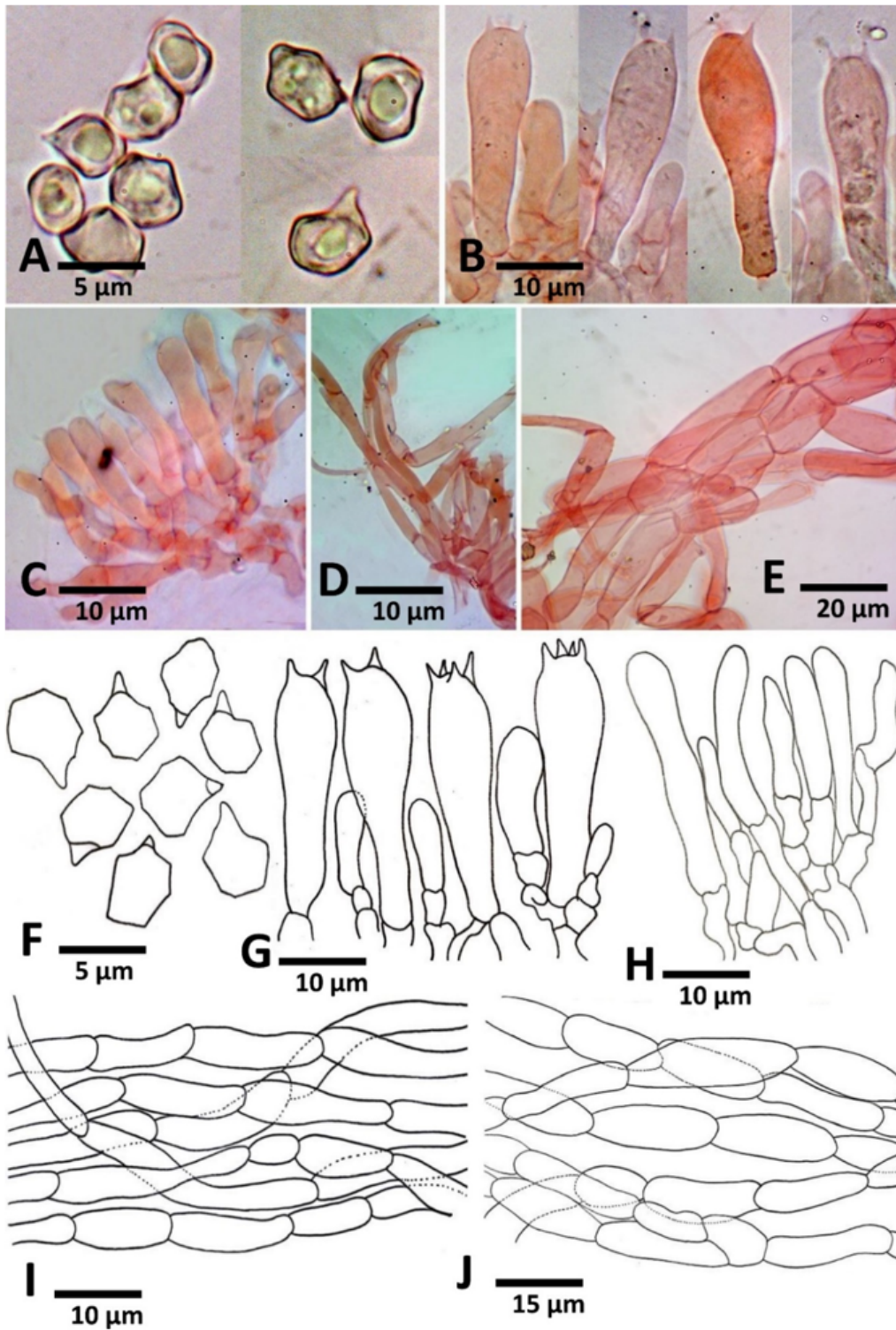


Figure 3

A-J: Microscopic features and Line drawings of *Entoloma khanspurenissp. nov.* (AY-31, holotype). A, F. Basidiospores, B, G. Basidia, C, H. Cheilocystidia, D, I. Stipitipellis, E, J. Pileipellis.

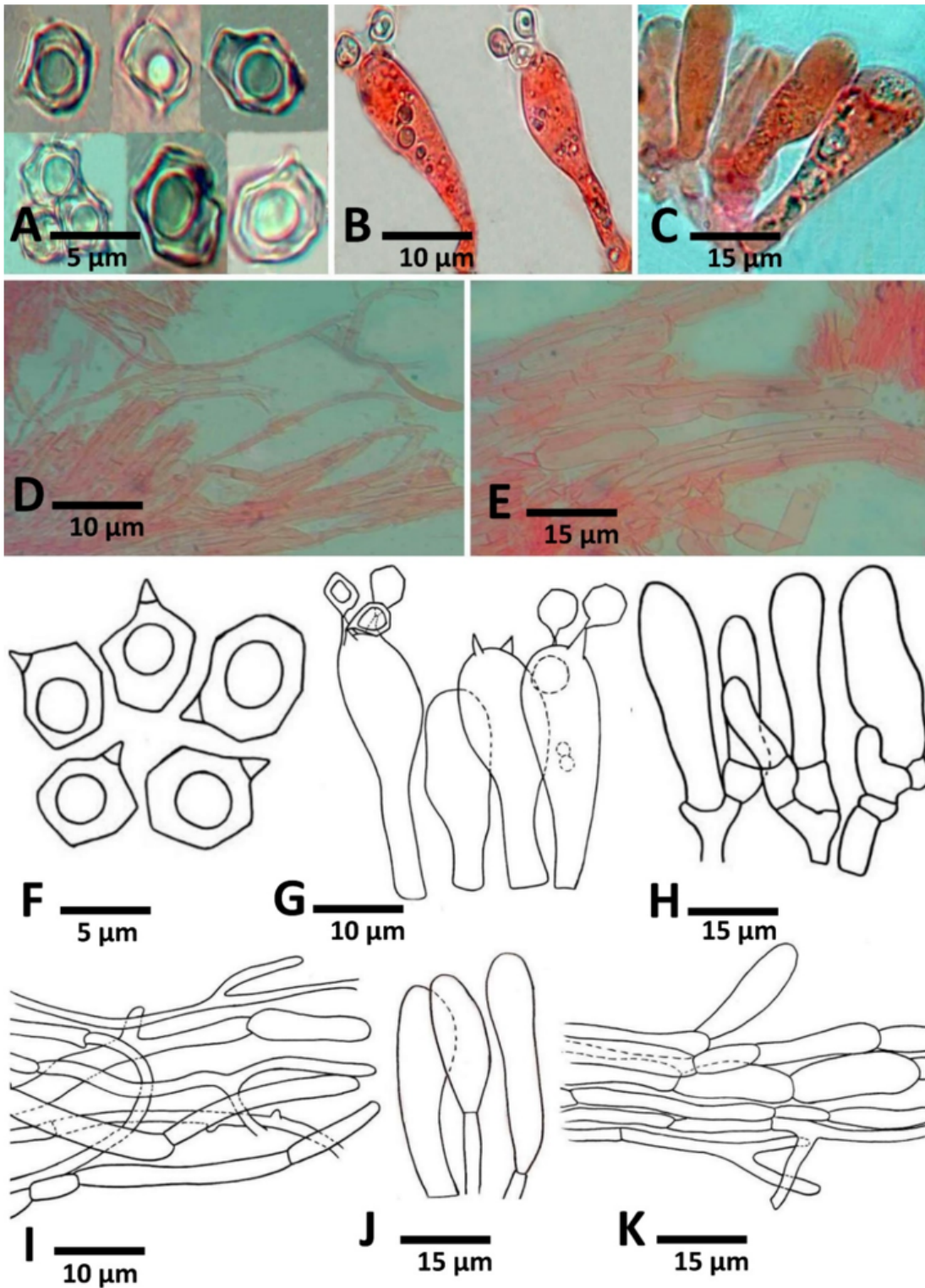


Figure 4

A-K: Microscopic features and Line drawings of *Entoloma pakistanicum* sp. nov. (ANK-252, holotype). A, F. Basidiospores, B, G. Basidia, C, H. Cheilocystidia, D, I. Stipitipellis, E, K. Pileipellis, J. Terminal elements.

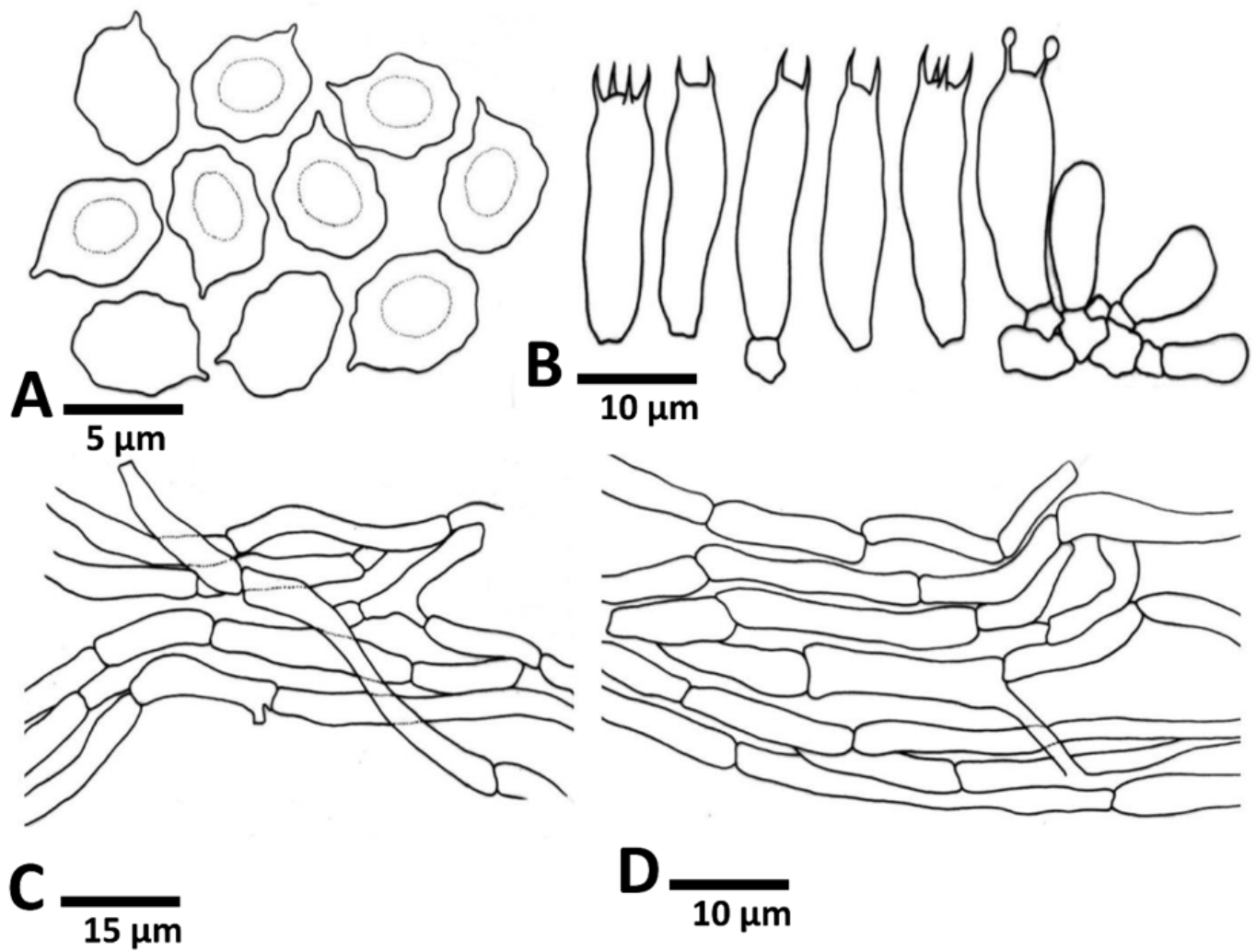


Figure 5

A-E: Line drawings of *Entolomaalbotomentosum* (B6, paratype). A. Basidiospores, B. Basidia, C. Pileipellis, E. Stiptipellis.

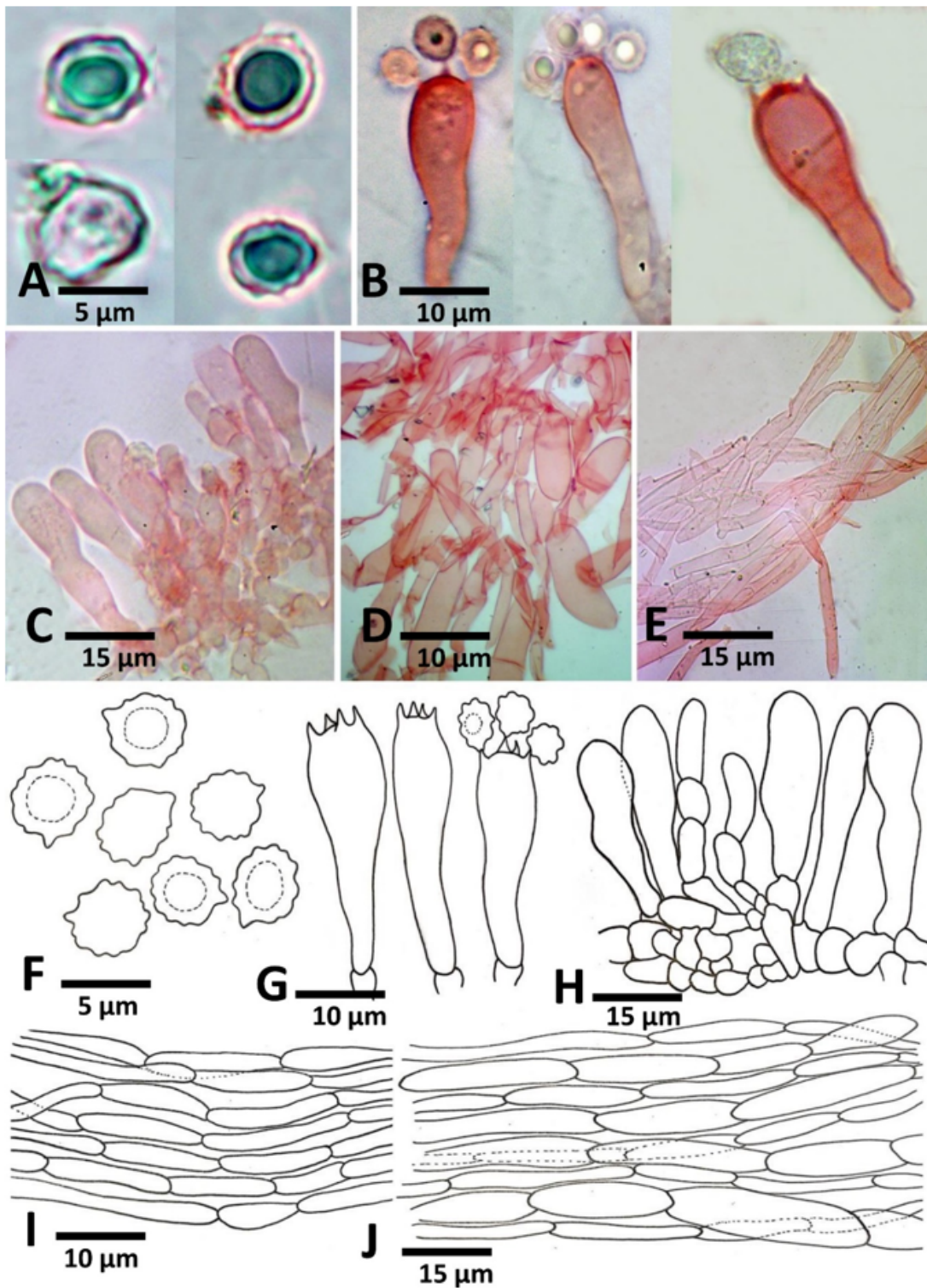


Figure 6

A-J: Microscopic features and Line drawings of *Rhodophana margallensis* sp. nov. (MH-34, holotype). A, F. Basidiospores, B, G. Cheilocystidia, C, H. Basidia, D, J. Pileipellis, E, I. Stipitipellis.