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Two new species of Entoloma (Agaricales, Basidiomycota) from Khyber Pakhtunkhwa, Pakistan.

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Abstract

Cedar towering forests of Kumrat valley, Khyber Pakhtunkhwa, Pakistan were visited to collect mushrooms during several fungal forays, which revealed two interesting and novel species from genus *Entoloma,* subgenus *Cyanula*, both are characterized by clamp-less hyphae and bluish-violaceous tinges on their pilei and stipes. *Entoloma kumraticus* is characterized by its bluish brown centrally depressed pileus, serrulate lamellar edge, bluish violet fibrillose stipe, dense clusters of cheilocystidia and presence of abundant greyish brown intracellular pigments in terminal cells of pileipellis and in few cheilocystidia. The key characters of *Entoloma swatica* are light brownish olive pileus with violet-blue finely fibrillose covering, translucently striated with radial stripes, blackish brown lamellar edge with clusters of cheilocystidia which are often septate and scaly, fibrillose slightly twisted violet blue stipe. Both new species are described and illustrated here, based on morphological and molecular evidence. *E. kumraticus* and *E. swatica* formed distinct phylogenetic lineages based on the sequences of nuclear ribosomal ITS and LSU gene regions.

Introduction

The species-rich agaric genus *Entoloma* (Fr.) P. Kumm. is well known for its pink spore print and angularity of basidiospores in both side and end views (Noordeloos 1980, Noordeloos 1981; Noordeloos 1984; Singer 1986; Gates and Noordeloos 2007; Noordeloos and Hausknecht 2007; Noordeloos and Morozova 2010; Ediriweera et al. 2017; Wartchow and Braga-Neto 2019; Brandrud et al. 2020; Noordeloos et al. 2021). Members of family Entolomataceae Kolt. & Pouzar are cosmopolitan, reported from arctic, semiarid, temperate wet tropical regions as well as from Europe, where recently dozens of new species have been described (Pegler 1977; Largent 1994; Manimohan et al. 1995, 2006; 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). Most of the species are saprophytic on litter and soil, whilst few are ectomycorrhizal such as *E rhodopolium* (Fr.) P. Kumm., associated with angiosperms and conifers (Co-David et al. 2009; Ediriweera et al. 2017).

Genus *Entoloma* has been splitted into several sub-genera including *Entoloma* sensu stricto, *Claudopus*, *Cyanula*, *Inocephalus*, *Leptonia*, *Nolanea*, *Pouzarella*, *Richoniella* and *Trichopilus* (Largent 1994; Noordeloos and Gates 2012). Species from *Entoloma* subgenus *Cyanula* are characterized by their colorful finely squamulose pilei, serrulatum-type (entirely sterile) lamellae edge, bunches of clavate, cylindric or vesiculose cheilocystidia, filled with purple, blue, brown to blackish intracellular pigments, frequent refractive oil droplets or brilliant granules in pileus and trama of the gills and absence of clamp connections mostly (Noordeloos and Liiv 1992; Noordeloos 2004; Noordeloos and Gates 2012). More than 2500 species of *Entoloma* have been described world-wide (Romagnesi 1941; Romagnesi and Gilles 1979; Horak 1980, 1982, 2008; Noordeloos and Liiv 1992, Noordeloos 2004; Largent 1994; Baroni and Halling 2000; Karstedt et al. 2007; Co-David et al. 2009; Henkel et al. 2010; Kasuya et al. 2010;; Lorås and Eidissen 2011; Noordeloos and Gates 2012; He et al. 2013; Karstedt and Capelari 2013, 2015, 2017, 2019; Lorås et al. 2014; Morozova et al. 2014; Weholt et al. 2014, 2015, 2016; Ediriweera et al. 2017; Brandrud et al. 2018; Crous et al. 2017, 2019; Noordeloos et al. 2018; Noordeloos et al. 2021; Dima et al. 2021; Reschke et al. 2022) but only eight species have been reported from Pakistan (Ahmad 1962; Ahmad et al. 1997). Here we describe two new members of *Entoloma* subgenus *Cyanula* based on detailed taxonomy and phylogenetic affinities by analyses of combined nrITS (internal transcribed spacer) and nrLSU (large ribosomal subunit) gene regions.

Materials And Methods

Sampling site

The Kumrat valley is geographically located between 35° 31'41.03" N, 72°14'06.47" E in Dir upper district, Khyber Pakhtunkhwa, Pakistan (Ahmad et al. 2014; Arif et al. 2015). This scenic valley is famous for its giant mountains, towering cedar forests and waterfalls. The common vegetation cover of these from moist temperate forests includes *Picea smithiana* Boiss., *Taxus baccata* L., *Cedrus deodara* (Roxb. ex D. Don) G. Don, *Pinus walllichiana* A. B. Jacks., scattered among mosses (Rajpar et al. 2020).

Macro- And Micromorphological Studies

All collections were photographed at the collection site. Field notes were prepared just after collection to preserve the ephemeral macroscopic characters. Notes were also made related to surrounding ecology and vegetation. Color notations were derived from Color Standards and Color Nomenclature (Ridgway 1912). The specimens were dried in front of a fan heater. After micro-morphological analyses and molecular studies, all material was deposited at Lahore Herbarium, Institute of Botany (LAH), Pakistan. Microscopic characters were examined from dried sections mounted in distilled water (to observe pigments), KOH solution (5%), 1% Congo Red and Melzer`s reagent (for any amyloid or dextrinoid reactions) in the laboratory. Photographs of microstructures were taken using a trinocular OLYMPUS CH30 microscope and ScopeImage 9.0 was used for microscopic measurements. At least 40 readings of each part i.e., basidia, cystidia, basidiospores, elements of pileipellis and stipitipellis per collection were measured. The dimensions of basidiospores are presented as (a) b – c (d), where a = extreme minimum value, d = extreme maximum value, b–c = range between 5th percentile and 95th percentile, Q = spore length: width individually, and avQ = Mean of length/width, for both spore’s length and width respectively. The following abbreviation [n/m/p] shows n = number of basidiospores from m = basidiocarps, of p = collection, with at least 20 basidiospores from each collection.

Molecular Study

From dried basidiomata, genomic DNA was extracted following Bruns (1995). PCR amplification was done for internal transcribed spacer region and LSU region of nuclear ribosomal DNA. The primer pairs ITS1F + ITS4 for ITS region and LROR + LR5 for LSU region were used in both PCR reactions and sequencing process for barcoding of ITS and LSU region of nrDNA (Gardes and Bruns 1993; White et al. 1990;). Bidirectional sequencing for each region was performed from TsingKe, China commercially. All sequences generated in our study are deposited and accessioned by GenBank.

Sequence Alignment And Phylogenetic Reconstruction

Sequence chromatograms were checked, both forward and reverse sequences were assembled, and consensus sequences for each region were generated using BioEdit v. 7.0.9.0. (Hall 1999). The consensus sequences were run on nucleotide BLAST search tool of GenBank for closest matches. Sequences showing maximum identity were retrieved from NCBI along with sequences from recently published literature (He et al. 2017 Morozova et al. 2018; Noordeloos et al. 2021; Reschke et al. 2022). Our combined ITS + nLSU dataset (Fig. 1, Table 1) included 54 fungal sequences including two outgroup taxa (*Clitopilus cystidiatus* Hauskn. & Noordel. and *C. hirneolus* (Fr.) Kühner & Romagn) following He et al. (2017). Final data set was aligned by online tool MUSCLE ver. 3.7, then alignment was manually adjusted in BioEdit. All positions with missing data and gaps were excluded. The final aligned dataset was analyzed through RAXML–HPC2 v 8.1.11, on CIPRES portal ver. 3.1 (www.phylo.org; Miller et al. 2010). Maximum Likelihood (ML) was inferred by selecting GTRCAT model, branch support values were calculated from 1000 BS replicates. The final tree was visualized in FigTree ver. 1.4.3. (http://tree.bio.ed.ac.uk/software/figtree/).

Table 1
A list of species, geographical origins, voucher numbers, GenBank accession numbers, and references of taxa used for the combined nrITS and nrLSU based phylogenetic analyses. Sequences generated from this study are shown in bold.

Species	**Origin**	**Voucher**	**GenBank accessions**		**References**
			ITS	**LSU**	
Entoloma aff. necopinatum	Panama	KaiR646	MZ611670	MZ678748	Reschke et al. 2022
Entoloma arcanum	Panama	KaiR614		MZ678739	Reschke et al. 2022
Entoloma arcanum	Panama	KaiR488		MZ678738	Reschke et al. 2022
Entoloma caeruleomarginatum	Panama	CME3	MZ611627		Reschke et al. 2022
Entoloma caeruleomarginatum	Panama	KaiR535	MZ611658		Reschke et al. 2022
Entoloma cf. catalaunicum	Estonia	E163	UDB011680 (UNITE)		Liiv, unpublished
Entoloma cf. largentii	USA	OSC144006	KX574458		Gordon, unpublished
Entoloma cf. *pseudoturci*	Croatia	Cro16	MZ611633		Reschke et al. 2022
Entoloma cf. *unicolor*	USA	PBM3995	KY777373		Matheny et al., (Unpublished)
Entoloma cf. *violaceobrunneum*	Panama	KaiR632	MZ678740		Reschke et al. 2022
Entoloma cf. *violaceobrunneum*	Panama	CME2	MZ678741		Reschke et al. 2022
Entoloma coracis	Norway	O-F-256850	MW934571	MW934251	Crous et al. 2021
Entoloma fuscosquamosum	USA	MGW1508	KY744158		Matheny et al., (Unpublished)
Entoloma glaucobasis	Germany/Sweden	Both 16–6–95/NL-2704	MZ869021	MK277991	Reschke et al. 2022, Varga et al. 2019
Entoloma griseocaeruleum	Panama	CME8	MZ611631		Reschke et al. 2022
Entoloma griseocaeruleum	Panama	CME13	MZ611624		Reschke et al. 2022

Species	Origin	Voucher	GenBank accessions		References
			ITS	LSU	
Entoloma griseocyaneum	Germany	KaiR997	MZ611684		Reschke et al. 2022
Entoloma griseocyaneum	Canada	UWO:PO6 (MO 215259)	KY706188		Hay et al. 2018
Entoloma holmvassdalenense	Norway	O-F-304575	MZ869018	MZ678746	Reschke et al. 2022
Entoloma incanum	Germany	KaiR990	MZ611683		Reschke et al. 2022
Entoloma kumraticus	**Pakistan**	**LAH36945 (Holotype)**	**MZ157265**	**MZ157269**	**This study**
Entoloma kumraticus	**Pakistan**	**LAH36946**	**MZ157266**	**MZ157270**	**This study**
Entoloma longistriatum	USA	PBM4018	KY744164		Matheny et al., unpublished
Entoloma mediterraneense	Croatia	Cro26	MZ611634		Reschke et al. 2022
Entoloma melleosquamulosum	Panama	KaiR638	MZ611669		Reschke et al. 2022
Entoloma melleosquamulosum	Panama	CME16	MZ611626		Reschke et al. 2022
Entoloma microserrulatum	Panama	KaiR413,	MZ611642		Reschke et al. 2022
Entoloma microserrulatum	Panama	KaiR664	MZ611671		Reschke et al. 2022
Entoloma montanum	Norway/Norway	O-F-293389/N03-09–2010	MW340878	MZ678747	Noordeloos et al. 2021/ Reschke et al. 2022
Entoloma mougeotii	Russia, Caucasus	LE254352	KC898446		Morozova et al. 2014a
Entoloma nigrovelutinum	Vietnam	LE295077	MF898426	MF898427	Crous et al. 2017
Entoloma nipponicum	Japan	TNS-F70747	MK693223	MK696392	Crous et al. 2019
Entoloma nipponicum	Japan	TNS-F70746	MK693222	MK696391	Crous et al. 2019

Species	Origin	Voucher	GenBank accessions		References
			ITS	LSU	
Entoloma norlandicum	Norway	O-F-76176	MW340899		Noordeloos et al. 2021
Entoloma norlandicum	Norway	O-F-76177	MW340900		Noordeloos et al. 2021
Entoloma ochromicaceum	Estonia/Denmark	TUF120040/DMS-9201008	Unite: UDB023715	MZ678743	Liiv, unpublished/ Reschke et al. 2022
Entoloma odoratum	Denmark	DMS-166826	MZ869017	MZ678745	Reschke et al. 2022
Entoloma porphyrogriseum	Sweden	G0207		MK277960	Dima et al. 2018, unpublished
Entoloma querquedula	Finland	18.XI.2011 TUR	LN850627		Kokkonen 2015
Entoloma roseotinctum	Norway	JL-26–19	MZ869019		Reschke et al. 2022
Entoloma sarcitulum	Great Britain	K378	LN850561		Kokkonen 2015
Entoloma serrulatum	Russia	LE254361	KC898447		Morozova et al. 2014
Entoloma subcaesiocinctum	China	GDGM31059	KY972699		He et al. 2017
Entoloma subcoracis	Russia	LE312483	MW934593	MW934255	Crous et al. 2021
Entoloma subfarinaceum	USA	SAT1518702	KY777374		Matheny et al., unpublished
Entoloma subserrulatum	Canada	EL9	KY706167		Hay et al. 2018
Entoloma swatica	**Pakistan**	**LAH36947 (Holotype)**	**MZ157271**	**MZ157267**	**This study**
Entoloma swatica	**Pakistan**	**LAH36948**	**MZ157272**	**MZ157268**	**This study**
Entoloma turci	Germany	WPR004	MZ611693		Reschke et al. 2022

Species	Origin	Voucher	GenBank accessions		References
			ITS	LSU	
Entoloma viiduense	Estonia	G1602	UDB015211	MK278008	Liiv, unpublished/ Varga et al. 2019
Entoloma violaceoserrulatum	Finland	TUR JV 8329F	MF476913	MF487803	Morozova et al. 2017
Entoloma yanacolor	Ecuador	QCAM6312	MG947210		Crous et al. 2018
Outgroups					
Clitopilus cystidiatus	Switzerland	TO AV130	HM623129	HM623132	Vizzini et al.2011
Clitopilus hirneolus	Italy	MEN 199956	KC710132	GQ289211	Morozova et al. 2014

Results

Phylogenetic analyses

Sequences of ITS and LSU gene regions of nrDNA from two collections of *E. kumraticus*; K-218 (LAH36945), K-219 (LAH36946), and *E. swatica* KU-74 (LAH36947), KU-75 (LAH36948), were obtained. The final dataset had an aligned length of 1625 characters, of which 939 were conserved sites, 653 are variable and 492 were parsimony-informative. In maximum likelihood analyses, (Fig. 1), Pakistani species *E. kumraticus* appeared as monophyletic and this species is found sister to the clade containing *E. porphyrogriseum* Noordel. and *E. arcanum* Reschke & Noordel. However, our species appeared as a distinct species with strong support (94%). Similarly, two collections of our second species *E. swatica* appeared as monophyletic with a high bootstrap support (96%) and is sister to *E.* cf. *catalaunicum* (Singer) Noordel. The phylogram (Fig. 1) inferred from combined nrITS+nLSU sequences revealed that both new species represented distinct monophyletic lineages with high statistical support.

Taxonomy

Entoloma kumraticus A. Izhar, Kiran, Usman & Khalid *sp. nov.* (Figs. 2 and 3)

MycoBank: MB843782

Diagnosis: Pileus bluish brown, centrally depressed; serrulate lamellar edge; bluish violet, fibrillose stipe; dense clusters of cheilocystidia; greyish brown intracellular pigments abundant in terminal cells of pileipellis and in some cheilocystidia.

Type: PAKISTAN. Khyber Pakhtunkhwa province, Dir Upper district, Kumrat, 2232 m asl, in grassy places on soil under *Cedrus deodara*, 25 July 2019, A. N. Khalid K-218, (LAH36945!; GenBank accessions for ITS: MZ157265; for LSU: MZ157269)

Etymology: The specific epithet *kumraticus* refers to the type locality.

Description: *Pileus* 1.8–2.5 cm in diameter, hemispheric, convex to plano-convex, deeply depressed at disc, deflexed towards margins, margins entire to sulcate-striate, dark aniline blue (X55) at center changing to Prout`s brown (XV15') to mummy brown (XV17') towards margin, pileal surface radially fibrillose, dry, velutinous, shiny when moist, slightly hygrophanous. *Lamellae* adnate, slightly adnexed, sub-distant, white (LI III) to pallid purplish grey (LIII 67""), regular, some crisped, forked near margin, margins serrulate, lamellulae abundant, present in 2–3 tiers. *Stipe* 3.2–5 × 0.3–0.6 cm, cylindrical with slightly tapered base, equal, hollow, pale neutral grey ((LIII) to purplish grey (LIII 67"") at apex, pallid bluish violet (x57f) towards base, fibrillose, minutely pubescent all over, dry, some with white (LI III) tomentum at base. *Annulus* and *Volva* absent. *Odor* Mild.

Basidiospores [40/2/2], (8–)9–11(–12) × (6.1–)6.4–8 µm. avl × avw = 9.7 × 7.3 µm, Q = 1.25–1.38, avQ = 1.32, ellipsoid, heterodiametrical with 4–6 weak angles, thin-walled, olive yellow (5Y6/8) in KOH, inamyloid, mono-guttulate. *Basidia* (29–)33–47(47.3–) × (9–) 10.3–12.2(12.3–) µm. avl × avw = 38.9 × 11.6 µm, broadly clavate, hyaline in KOH, mostly 4-spored, some bi-spored, guttulate, clamp connections absent. *Lamella edge* sterile, serrulatum-type, made up of dense clusters of cheilocystidia, 12–34 × 4.9–8.4 µm, avl × avw = 19.5 × 6.5 µm, cylindrical to clavate, some flexuous, thin-walled hyaline in KOH, basal cells few aseptate others bi-tri septated, non-guttulate. *Hymenophoral trama* regular, composed of cylindrical elements, 80–125 × 4–9 µm. *Pileipellis* a cutis with transitions to a trichoderm, made up of inflated, clavate terminal elements, similar to pileocystidia, 30–93 × 15–25 µm, upper pileipellis cells 22–80 × 4–11 µm, a subpellis of relatively narrow septate, cylindrical hyphae, with 6–9 µm wide, avw = 7 µm, pigments intracellular as clusters, brown in water, relatively dark brown in KOH, clamp connections absent. *Stipitipellis* an intricate trichoderm, with cylindrical to clavate terminal elements, 1.6–5.8 µm wide, avw = 3.8 µm, mostly hyaline in KOH, others with dark brown brilliant granules, hyphae regular, septate, rarely branched, clamp connections absent. *Caulocystidia* absent.

Additional specimens examined. PAKISTAN. Khyber Pakhtunkhwa province, Dir Upper district, Kumrat, 2232 m asl, mostly solitary on moss covered soil under *Cedrus deodara*, 15 August 2018, M. Usman and A. N. Khalid K-219, (LAH36946!; GenBank accessions for ITS: MZ157266; for LSU: MZ157270)

Remarks

Entoloma kumraticus sp. nov. is conspicuous on account of its bluish brown centrally depressed pileus, greyish, serrulate lamellar edge, bluish violet, fibrillose stipe, dense clusters of clavate to cylindrical, septate cheilocystidia at lamellar edge, greyish brown intracellular pigments abundant in terminal cells of pileipellis and in some cheilocystidia.

Phylogenetically *E. kumraticus* formed a distinct clade in phylogenetic analyses, and based on molecular data available, it can be distinguished from all other members of subgenus *Cyanula*. On Molecular analysis, *E. kumraticus* is the closest relative to *E. porphyrogriseum* in the phylogenetic tree (Fig. 1), presented here separating with 94% bootstrap support. *E. porphyrogriseum* is a common grass land species from Austria, differs in having comparatively large diameter of pileus (up to 3.7 cm), pileus surface violet brown to black, a reddish coloration is present from center towards edges throughout the pileus, dirty pink to greyish pink lamellae, longer stipe reaching up to 7 cm getting greyish red towards base, short basidia (22–35 µm in length) and bigger cheilocystidia 25–41 × 6–13 µm (Noordeloos et al. 1995b). When nrLSU sequence of these two species were compared, the sequence of *E. porphyrogriseum* (MK277960) showed differences at 25 nucleotide positions.

Phylogenetically, *E. arcanum* seems to be another closely related species to *E. kumraticus* but morphologically *E. arcanum* differs from *E. kumraticus* due to its non-translucently striate and non hygrophanous pileus with large pileus diameter (3.0–4.5 cm), adnate to slightly decurrent lamellae, fusiform cheilocystidia and much larger end cells of pileipellis (40–140 × 10.5–19.0 µm) and was found under *Alnus*- and *Quercus*-dominated forests (Reschke et al. 2022).

The combined nrITS and LSU sequences based phylogram (Fig. 1) shows that *E. kumraticus* is related to *E. melleosquamulosum* Reschke, Manz & Noordel., but the later one differs morphologically by its honey-colored pileus with scaly surface, distinct scales at disc, sinuate, segmentiform and ventricose lamellae, pale yellow to white stipe and its habitat in tropical submontane forest covered by *Oreomunnea mexicana* (Standl.) J.-F.Leroy and *Quercus* species. While looking towards micromorphology *E. melleosquamulosum* produces thickwalled, yellowish pink pigmented basidiospores, smaller basidia (26–35 × 8.0–10.0 µm), significantly longer (23–64 µm), lageniform cheilocystidia, hymenophoral trama with much larger cells (65–200 × 4.0–19.0 µm), a cutis type stipitipellis and presence of oleiferous hyphae in the trama (Reschke et al. 2022).

E. kumraticus is close to a sequence (KY744158) labelled as E. *fuscosquamosum* Hesler. This American species, *E. fuscosquamosum* differs by its mouse gray pileus, white to pinkish, arcuate to sub-decurrent, lamellae, somewhat larger basidiospores (9–13 × 6–7.5 µm), smaller basidia (34–41 × 8–10 µm), significantly larger capitate cheilocystidia (32–54 µm in length), pileocystidia bigger with size range of 40–80 × 10–15 µm or complete absence of cheilocystidia ((Helser 1967; Noordeloos 1988).

E. fuscosquamosum was reported from humus rich soil of Cades Cove, Tennesse, which is a flat valley with temperate climate, present between smoky mountains, the dominant vegetation of the valley is *Quercus alba* L., *Tsuga canadensis* L., and *Pinus strobus* L. (Helser 1967; Noordeloos 1988). *E. kumraticus* has been collected from moist temperate forests where vegetation cover was including *Picea smithiana* Boiss., *Taxus baccata* L., *Cedrus deodara* Roxb., *Pinus walllichiana* A. B. Jacks., scattered among mosses (Rajpar et al. 2020).

Pakistani species *E. kumraticus* are close to *E. violaceoserrulatum* Noordel., but *E. violaceoserrulatum* contrasts by its violet black to greyish brown pileus, adnate to emarginate lamellae occasionally with bluish black spots, relatively longer but narrow (20–50 × 2.5–7.0 µm) cheilocystidia, pileipellis just a trichoderm and no significant clamp connections observed (Dima et al. 2021).

Japanese species: *E. nipponicum* is closer to our species but it differs in its light orange to greyish red with occasionally umbilicate centre, radially splitting with age, stipe pale orange or whitish to grey towards base, smaller basidia (25–39 × 7–10 µm) and much larger (32–63 × 7–18 µm) sublageniform or subfusiform cheilocystidia (Crous et al. 2019).

Hesler (1967) placed *E. serrulatum* (Fr.) Hesler, under the synonymy of *Leptonia serrulata* (Fr.) P. Kumm. originally described from North Carolina, Tennesse, France, Sweden, Scotland and England. In comparison with the Pakistani species, *E. serrulatum* differs by its non-hydrophanous pileus, crowded, emarginate lamellae, green to olivaceous or brown stipe, ellipsoid 5–7 angled basidiospores, larger basidia (24–53 × 8–15 µm), bigger cheilocystidia (25–100 × 3.5–20 µm) with blue intracellular pigment, much wider trama hyphae (7–16 µm) as well as terminal elements of pileipellis (8–40 µm) having bluish intracellular pigment (Hesler 1967; Pegler 1977).

Entoloma swatica A. Izhar and Khalid *sp. nov.* (Figs. 4 and 5)

MycoBank: MB843783

Diagnosis: Bluish brown centrally depressed pileus, greyish, serrulate lamellar edge, bluish violet, fibrillose stipe, dense clusters of clavate to cylindrical, septate cheilocystidia at lamellar edge, greyish brown intracellular pigment abundant in swollen terminal cells of pileipellis and in some cheilocystidia.

Type: PAKISTAN. Khyber Pakhtunkhwa province, Dir Upper district, Kumrat, 2232 m asl, in groups on grassy spots under *Cedrus deodara*, 30 July 2019, A. N. Khalid KU-74, (LAH36947!; GenBank accessions for ITS: MZ157271; for LSU: MZ157267)

Etymology: The specific epithet *swatica* refers to district Swat, the type locality.

Description: *Pileus* 1.5–3.2 cm in diameter, hemispherical to plano-convex, depressed at center, deflexed then involute margins, margins entire, translucently striate, initially light brownish olive (XXX 19") to Saccardo's umber (XXIX 17") dark dull violet-blue (XXIV 53*) near margins, pileal surface wholly covered with minute squamules, crowded thin fibrils at disc, dry, dull, slightly hygrophanous. *Lamellae* emarginate with decurrent tooth, ventricose, moderately distant, white to pale lobelia violet (XXXVII 61") with warm blackish brown (XXXIX 1'''), uneven, finely crenulate lamellar edge, lamellulae abundant, mostly in 1–2 tiers. *Stipe* 4.5–5.5 × 0.4 – 0.7 cm, cylindrical, slightly swollen at base, hollow, Vanderpoel's violet (XXXVI 55") at apex, dark Tyrian blue (XXXIV 47") towards base, fibrillose to twisted, finely pruinose in upper portion, scaly all over, not shiny, dry, white (LI III) tomentum at base. *Odor* and *taste* not recorded.

Basidiospores [40/2/2], (7.3–)9.6–10.9(–11) × (6.5–)6.9–8(–8.5) µm. avl × avw = 10.15 × 7.26 µm, Q = 1.1–1.3, avQ = 1.2, ellipsoid, heterodiametrical, with 5–7 angles, thick-walled, hyaline in KOH, inamyloid, mono-multiguttulate. *Basidia* 37–38.6 × 9–10 µm. avl × avw = 38 × 9.5 µm, clavate, hyaline in KOH, frequently 4-spored, rarely 2-spored, guttulate, clamp connections absent. *Lamella edge* heterogeneous, made up of dense clusters of cheilocystidia, (13–)14–21.8(–23) × (6.1–)6.8–11.7(–15) µm. avl × avw = 20.9 × 8.8 µm, clavate to subvesiculose, thin-walled hyaline, some with brownish intracellular pigment in KOH, basal cells septate, guttules present. *Hymenophoral trama* regular to intermixed, made up of cylindrical to swollen elements, 73–100 × 5–9 µm. *Pileipellis* a cutis with transition to a trichoderm, composed of cylindrical to clavate end cells, 15–55 × 6–17 µm, upper pileipellis cells 12–18 × 3–7 µm, a subpellis with narrow, septate hyphae 2.8–6.4 µm, avw = 4.5 µm, frequent intracellar pigments as clumps, pale yellowish brown in water, brown in KOH, clamp connections absent. a cutis, hyphae composed of narrow cylindrical to clavate cells, 15–27 µm, avw = 19 µm, terminal cells relatively inflated, hyaline in KOH, parallel, septate, frequently branched, clamp connections absent. *Caulocystidia* absent.

Additional specimens examined. PAKISTAN. Khyber Pakhtunkhwa province, Dir Upper district, Kumrat, 2232 m asl, in meadows under *Cedrus deodara*, 12 August 2018, M. Usman and A. N. Khalid KU-75, (LAH36948!; GenBank accessions for ITS: MZ157272; for LSU: MZ157268)

Remarks

This species can be recognized by the light brownish olive to violet-blue finely fibrillose, translucently striate pileus, blackish brown lamellar edge, scaly, fibrillose to twisted stipe, clusters of cheilocystidia, often septate and lack of clamp connections in all tissues.

In our combined ITS+LSU analyses (Fig. 1), *E. swatica* formed a monophyletic lineage with European species *E. catalaunicum* previously known as *Leptonia catalaunica* Singer. However, *E. catalaunicum* differs from *E. swatica* by having non-translucently striate and non-hygrophanous pileus, pink lamellae, 6-9 angled basidiospores, larger cheilocystidia (25–100 x 7–20 µm), relatively broader terminal elements of pileipellis (12–30 µm wide) and stipitipellis a trichoderm with frequent caulocystidia (Singer 1936; Noordeloos 1982). *E. norlandicum* is another closer species and the major differences of *E. norlandicum* from *E. swatica* include dark brown, non-translucently striate pileus, lamellar edge white to pinkish concolorous to lamellae, terminal elements of pileipellis bigger (80–120 × 6–20 µm) and presence of caulocystidia (Noordeloos et al. 2021).

Our phylogenetic dataset shows that *E. swatica* is closer to *E. roseotinctum* Noordel. & Liiv, but *E. roseotinctum* is different on account of its greyish pink, non-hygrophanous and non-translucently pileus, pink-colored free lamellae and grey stipe. Microscopic features that make *E. roseotinctum* different from *E. swatica* are relatively slender and much longer lageniform cheilocystidia (25–60 × 8–13 µm) and bigger terminal cells of pileipellis (30–70 × 12–20 µm) (Noordeloos and Liiv 1992).

Entoloma swatica has a morpho-anatomical resemblance to phylogenetically closer species *E. largentii* Courtec., previously named *Leptonia convexa* Largent based on a fibrillose violet pileus covered with squamules, ventricose lamellae, purplish fibrillose stipe with a white base and ellipsoid basidiospores. However, *E. largentii* is easily distinguished from our species because of the sinuate lamellae, smaller basidiospores (8–10 × 5–6 µm), absence of cystidia and pale to violet pigmentation in the pileus hyphae (Courtecuisse 1986). When nr ITS sequences were compared, our DNA sequences were different at 47 nucleotide positions when compared with sequences of *E. largentii*.

E. holmvassdalenense Eidissen, Lorås & Weholt, differs due to non-striate margins of the pileus, a white lamellae edge, large size of basidiospores (10.2–13.7 × 7.8–10.9 µm), (1–)2-spored basidia, cheilocystidia somewhat lageniform, others with a swollen base and attenuated, mucronate or rostrate. *E. holmvassdalenense* has been reported from calcareous spruce forests of Holmvassdalen, Nature Reserve in Norway, where soils are roofed with tall to low herbs and some localities are covered with mosses like *Sphagnum* L. spp. (Weholt et al. 2014). In contrast to *E. holmvassdalenense,* our species was collected from moist temperate forests, from soil covered with coniferous trees.

When morphology was compared *E. caesiellum* Noordel. & Wölfel, appeared closer to our new species but *E. caesiellum* is different due to its brown to beige pileus covered with blackish blue granules, longer (up to 7.5 cm), glabrous stipe and pale yellowish intracellular pigments in the terminal cells of the pileipellis (Noordeloos 1995a; He et al. 2016).

A SriLankan species, *E. gnophodes* (Berk. & Broome) E. Horak, can be confused with *E. swatica* but *E. gnophodes* produces bigger basidiospores (10–13 × 7.5–10 µm), lack of cystidia and pileipellis repent cutis type (Pegler 1977).

Discussion

In the traditional classification of Entolomatoid species, both new species would be placed in subg. *Cyanula* strips Serrulatum, owing to morphological characteristics, such as bluish basidiomata and clampless tissues. Furthermore, our BLASTn search results and phylogenetic analyses based on combined nrITS and LSU sequences revealed that *E. kumraticus* and *E. swatica* were nested well in the clade dominated by the members of sub-genus *Cyanula*, with distinct lineages.

Declarations

Ethics Declaration

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 Muhammad Usman collected field samples. Aiman Izhar contributed the morphological and molecular analyses under the guidance of Abdul Nasir Khalid. Munazza Kiran contributed to the study conception and design. The first draft of the manuscript was prepared by Aiman Izhar and carefully revised by all authors.

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Figures

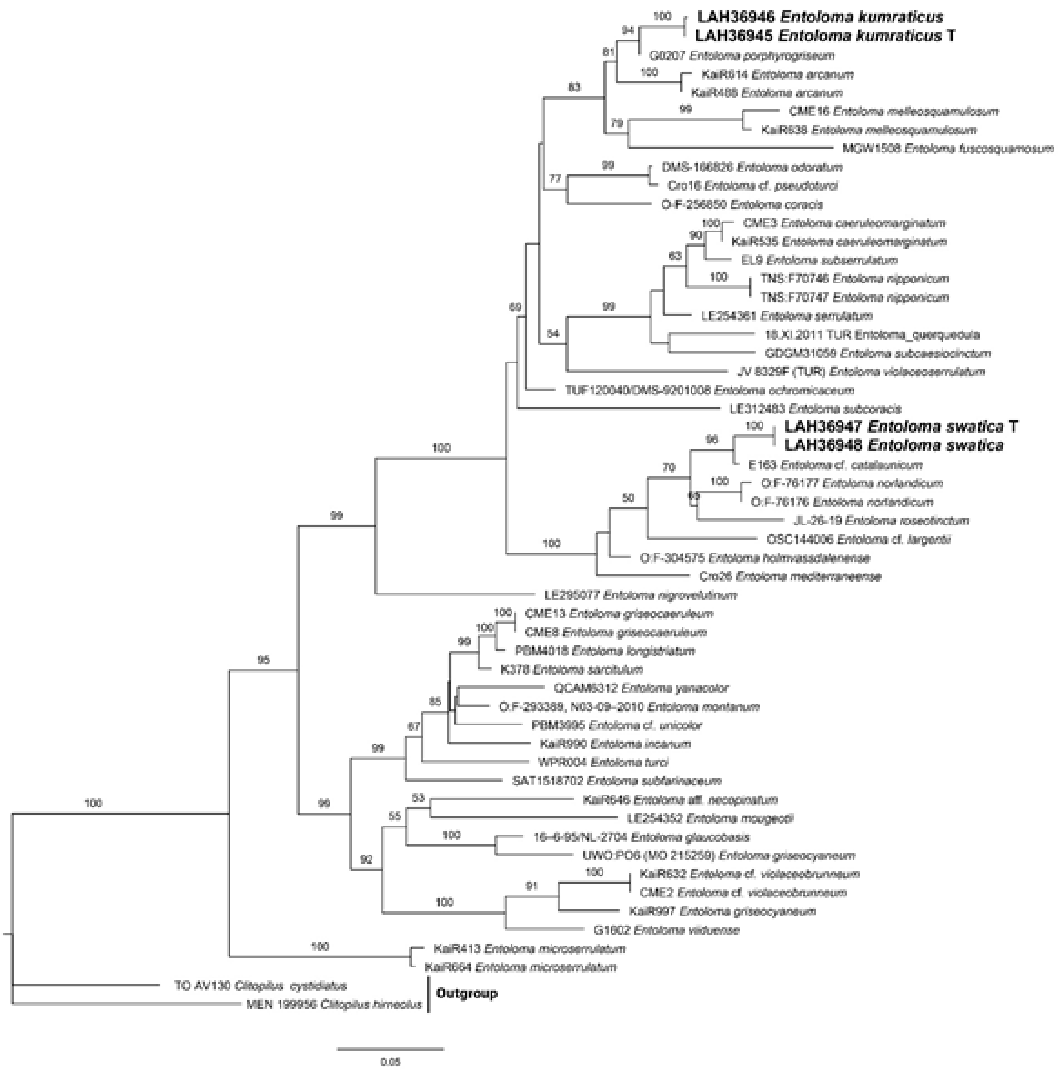

Figure 1

Molecular phylogenetic analysis of *Entoloma kumraticus* and *E. swatica* along other species of subgenus *Cynaula* by Maximum Likelihood (ML) method based on combined ITS and LSU sequences.

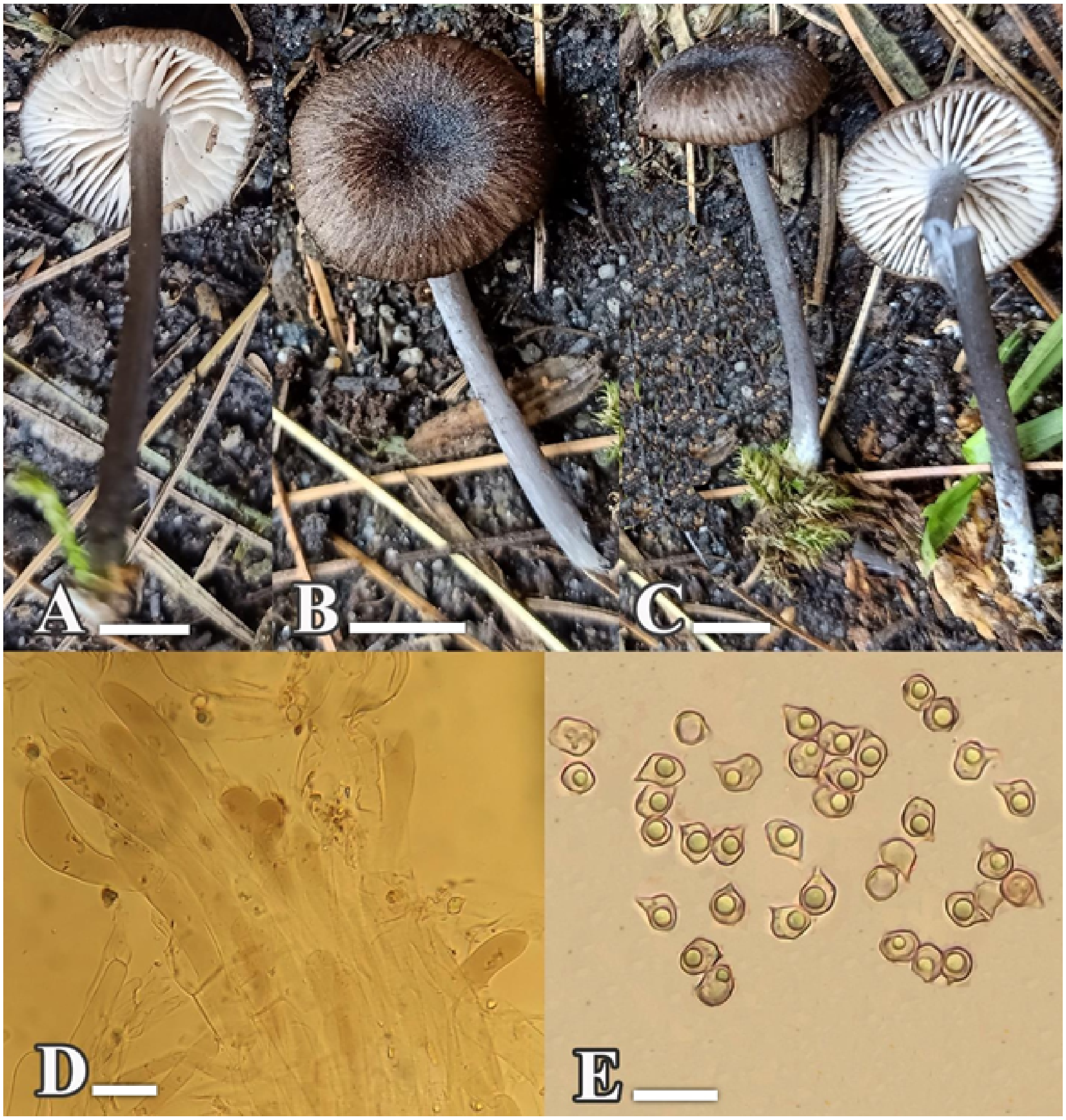

Figure 2

A–**C:** Basidiomata of *Entoloma kumraticus*; **D:** Pileipellis, **E:** Basidiospores; Scale Bars **A**–**C** = 1 cm, **D** = 20 μm, **E** = 10 μm. Microscopic photos by: Aiman Izhar.

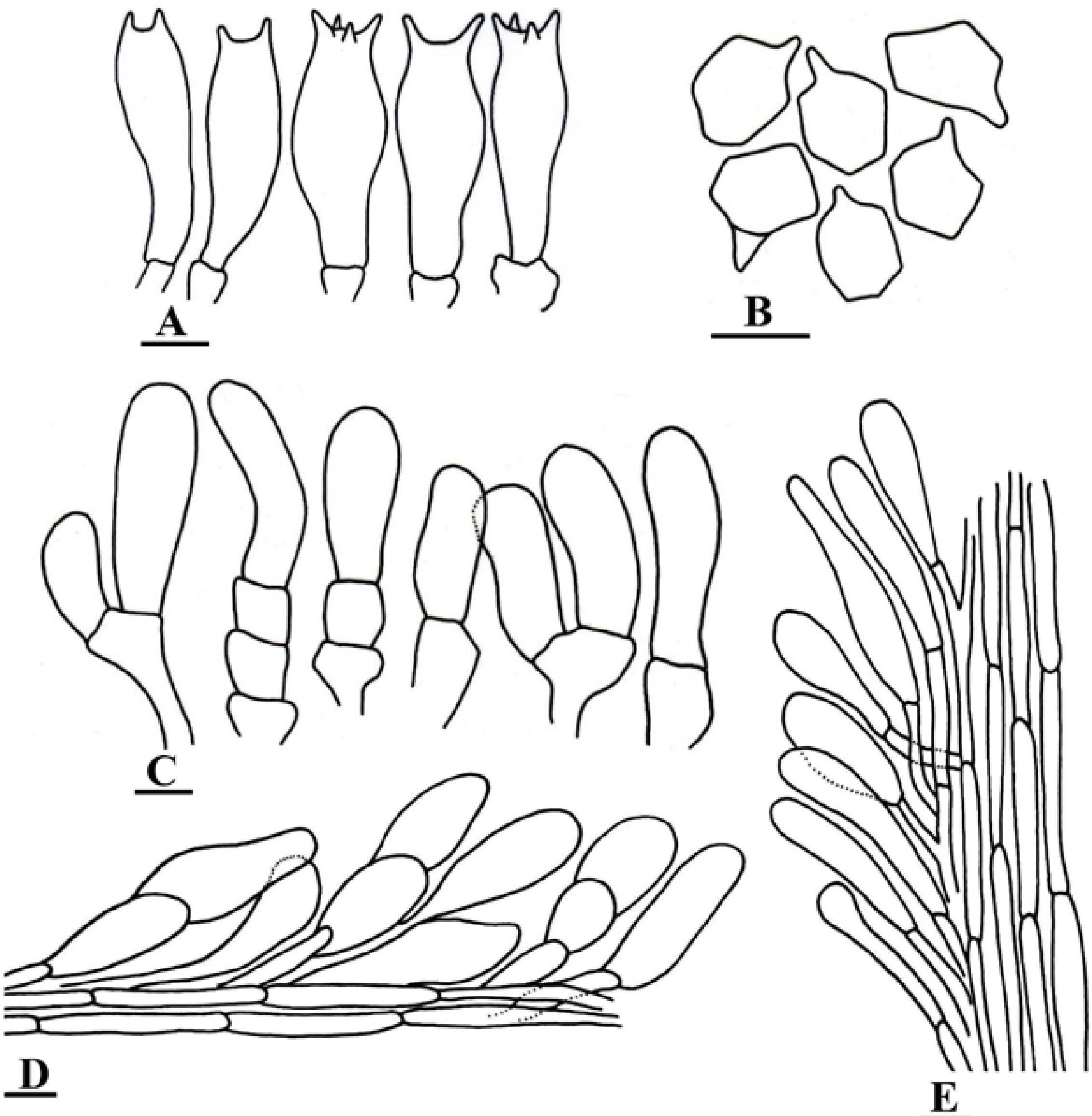

Figure 3

A–**E**. Microscopic characters of *Entoloma kumraticus* (Holotype). **A:** Basidia, **B:** Basidiospores; **C:** Cheilocystidia; **D:** Pileipellis; **E:** Stipitipellis; Scale Bars **A**–**E** = 10 µm. Drawings by: Aiman Izhar.

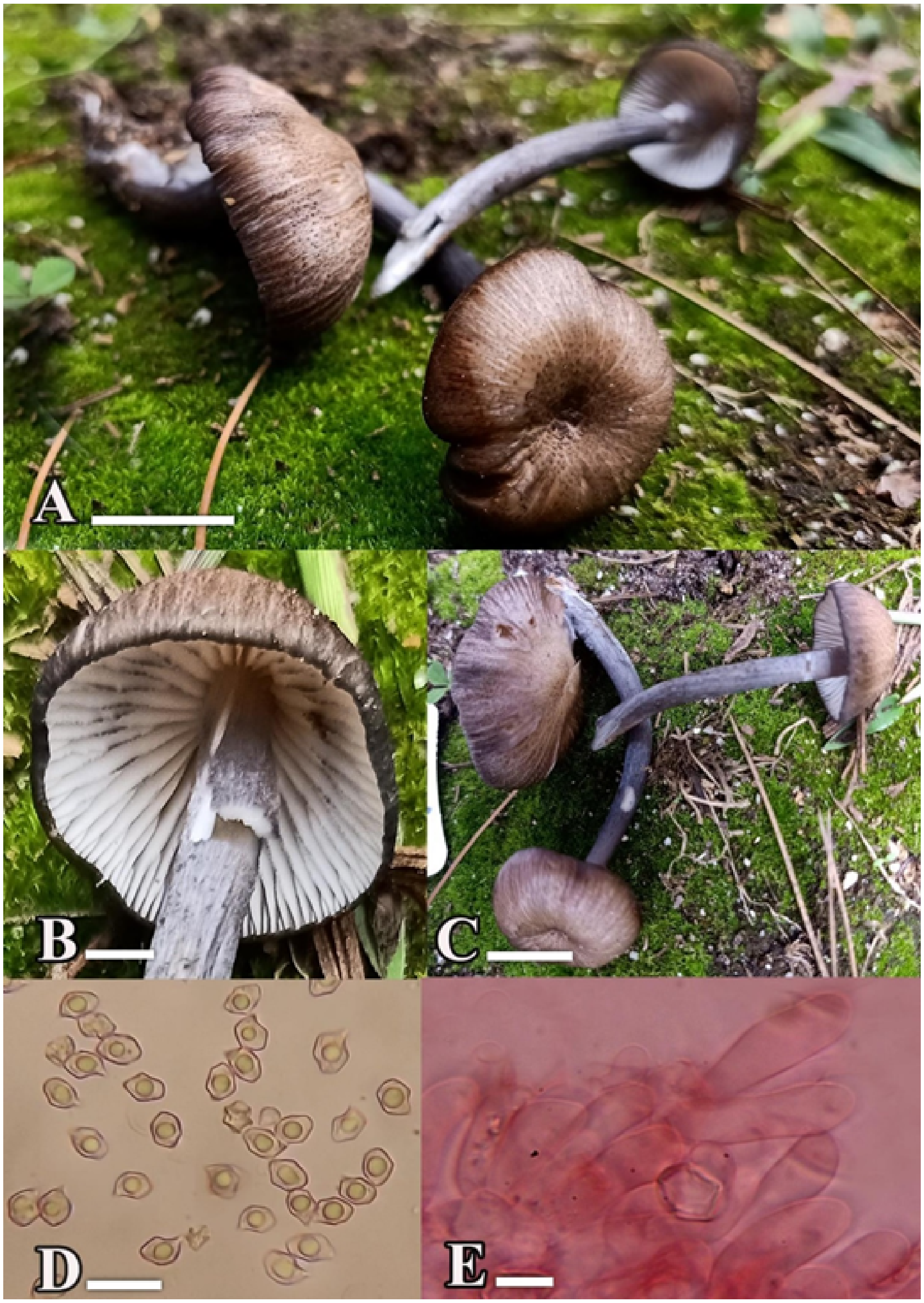

Figure 4

A–**C:** Basidiomata of *Entoloma swatica*; **D:** Basidiospores, **E:** Cheilocystidia, Scale Bars **A**–**C** = 1 cm, **D**, **E** = 10 μm. Microscopic photos by: Aiman Izhar.

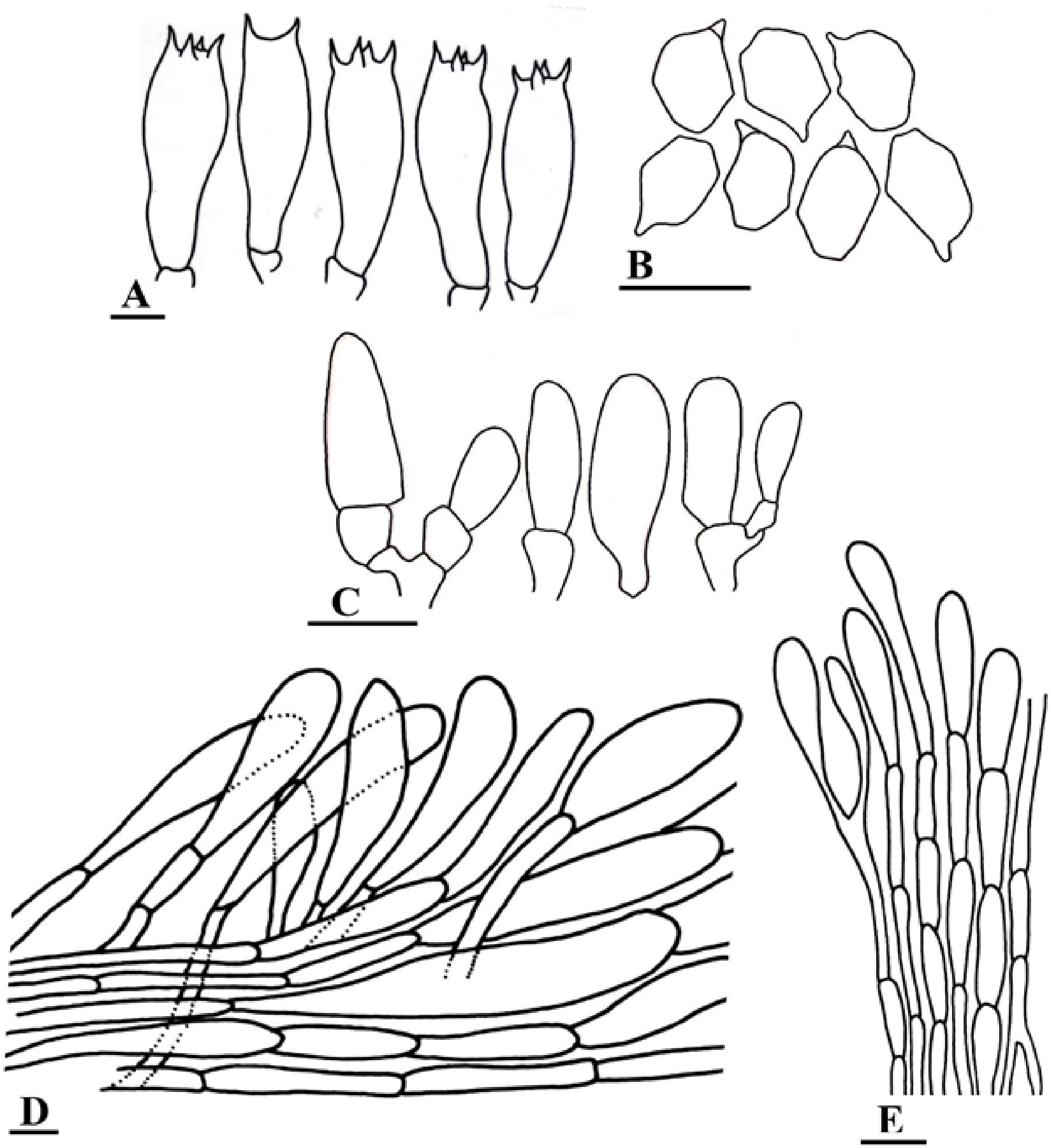

Figure 5

A–**E**. Microscopic characters of *Entoloma swatica* (Holotype). **A:** Basidia, **B:** Basidiospores; **C:** Cheilocystidia; **D:** Pileipellis; **E:** Stipitipellis; Scale Bars **A**–**D** = 10 μm, **E** = 20 μm. Drawings by: Aiman Izhar.