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FIRST REPORT OF MILKY CONE CAP (*CONOCYBE APALA*) FROM PUNJAB, PAKISTAN

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Abstract

During conduction of fungal surveys to record fungal biodiversity from plain areas of Punjab, several specimens of the genus *Conocybe*, were collected from four different areas with arid to semi-arid climate. The collections were described and identified as *Conocybe apala* on the basis of morphological and anatomical characters. Morphological plates of all collections, micrographs, and comparison to allied *Conocybe* species are provided.

Key words: Gujranwala, new record, taxonomy, white dunce cap

Introduction

Conocybe Fayod belongs to family Bolbitaceae of order Agaricales. It is morphologically characterized by the occurrence of tiny to medium-sized, plicate-sulcate edges on the basidioma, and microscopically by dark spores with a prominent germ-pore and lecythiform cheilocystidia with a circular capitellum. Round capitellum in cheilocystidia and ill-defined pileal margins are the main distinguishing features that separate *Conocybe* from other genera (Arnolds, 2005; Hausknecht and Krisai-Greilhuber, 2006; Amandeep *et al*., 2015). There are more than 450 species of *Conocybe* reported worldwide (Demirak *et al*., 2021). Members of this genus are commonly referred as dunce caps owing to their conical or bell-shaped crowns. *Conocybe* species are coprophilous and saprobic which prefer to grow on meadow, dung, pasture, parks, golf resorts, leaf litter, and woodchips either solitary or in clusters (Pegler, 1983, 1986, 1997; Singer, 1986; Latha *et al*., 2018; Liu and Bau, 2018; Halbwachs and Bassler, 2020). Members of this genus are prevalent in subtropical and tropical climates and commonly found in wet seasons. Majority of species occur in European countries, (Watling and Hausknecht, 1997; Hausknecht, 1998; Horak and Hausknecht, 2002; Hausknecht, 2009; Hausknecht *et al*., 2009; Malysheva, 2017), with few reports from Asia, i.e. China (Watling, 1971, Enderle, 1994, Enderle and Hübner, 1999, Melo *et al*., 2016; Liu and Bau, 2018), India (Amandeep *et al*., 2013; 2015), Pakistan (Izhar *et al*., 2019), and Turkey (Akçay *et al*., 2018; Çagli *et al*., 2019).

In the field, *Conocybe* species may be misidentified as *Galerina* Earle taxa and vice versa however, these can be distinguished by microscopic examination of pileus cuticle. The cuticle of cap is thread-like in the genus *Galerina* whereas, cobblestone-like in *Conocybe* (Amandeep *et al*., 2015).

The genus is economically significant because it contains a variety of secondary metabolites of enormous potential (Benedict *et al*., 1962; Benedict *et al*., 1967). A number of species are also known to create cyclic peptides, specifically amatoxins, as well as psilocybin and other

hallucinogens (Benedict *et al*., 1962, Benedict *et al*., 1967, Brady *et al*., 1975, Hallen *et al*., 2003).

Previously, several species of basidiomycetes are reported from plain areas of Pakistan (Izhar *et al*., 2019; Razaq *et al*., 2022). Further, on the basis of morphological characterization, nine species of genus *Conocybe* such as *C. khasiensis* (Berk.) Watling, *C. macrocephala* Kühner and Watling, *C. mesospora* Kühner and Watling, *C. pubescens* (Gillet.) Kuhner, *C. punjabensis* (Izhar), *C. rickenii* (Jul. Schäff.) (Kühner), *C. semiglobata* (Kühner and Watling), *C. semiglobata* var. *campanulata* Hauskn. and *C. tenera* (Schaeff.) (Fayod), have been recorded from subtropical regions of Pakistan (Ahmad *et al*., 1997; Izhar *et al*., 2019). The main objective of this study was to explore the diversity of mushrooms from unexplored regions of Punjab, and to find new fungal records for Pakistan.

Materials and Methods:

Sampling sites & Collection of samples

A total eleven collections of the taxon were made during years 2020-2022, from four different localities of Punjab province, nine collections from different sites of district Gujranwala, one collection from district Khanewal, and one from district Kasur. Specimens were photographed from different angels, labeled and field notes were prepared. Fruiting bodies were dried using fan heater. Fully dried and properly labeled specimens were kept in separate zip lock bags or paper boxes for further investigation.

Morpho-anatomical analysis

Morphological characterization of fresh specimens was done according to Thomas *et al*. (2001), Manimohan *et al*. (2007), Malysheva *et al*. (2015). After drying, the samples were brought to the lab for their microscopic analysis. Microscopic observations were made on dried samples under compound microscope (BioBlue and LABOMED). Sizes of basidia, cheilocystidia, basidiospores and hyphal elements for pileipellis, stipitipellis were measured with the help of ocular micrometer. Slides were prepared by using lactic acid, and 5% KOH solution as mounting media. 1% aqueous Congo Red solution, and trypan blue were used as staining agents. For descriptions of microscopic features, standard literature was followed (Pegler, 1977; Singer, 1986; Atri, 2005; Malysheva *et al*., 2015). Illustrations were also drawn with the help of camera Lucida. Micrographs were also captured using Andriod smartphone (Oppo Reno 3) attached with a compound microscope.

Results

Taxonomy

Conocybe apala (Fr.) Arnolds, Persoonia 18(2): 225 (2003) (Figs. 1-4)

Macroscopic Characterization

Basidiocarp 7.5–10 cm, soft and fleshy, epigeous, solitary to gregarious. **Pileus** 2.5–4 × 2–3 cm, initially conical, becoming broadly conical to bell shaped with age, surface greyish and slightly orange towards center, finally uplifted, with central obtuse umbo, often on a central shallow depression, glabrous, sulcate-striate to the center, glutinous when moist especially towards the center, flattened to reflexed from margins, becoming fissile or eroded, fleshy. **Lamellae** adnexed to almost free, pale at first then cinnamon brown, thin, close to crowded, unequal in arrangement, lamellae of three lengths, pale edges, non-deliquescent. **Stipe** 7–8.5 × 0.3–0.5 cm, central, surface white, cylindrical or slightly tapering and without substantial furrows and ridges, with scattered tiny hairs, thin towards apex, hollow, base slightly swollen, equal. **Volva** absent.

Partial veil absent. **Rhizomorphs** absent. **Odor** not recorded.

Microscopic Characterization

Basidiospores 11–17 × 5–8 µm, Q = 1.3–2.4, avg Q = 1.7, oblong, greenish, oil droplet present, ornamentation present, apiculus present, thick walled, rough. **Basidia** 28–42 × 7–20 µm, clavate or oblong, hyaline in 5 % KOH, attached with hymenium, bi-sterigmate, thin walled, without oil droplets. **Cheilocystidia** 17.0–28.2 × 12.0–22.7 µm, lecythiform with a sub-globose head 3–4 µm wide, a narrow (2.0 µm) neck, and an obclavate bottom portion, hyaline in 5 % KOH, thin walled, smooth. **Pileipellis** hyphae, 2.85–5.7 µm, septate, hyaline, thin walled, double layered, branched, bent or curved, interwoven, narrow-wide. **Stipitipellis** 4.2–5.7 µm, aseptate, hyaline, unbranched, thick walled, straight. **Clamp connection**s absent. **Pileipellis elements** 14–45 × 11–34 µm, oblong, double layered, euhymeniderm, hyaline, thin walled, smooth.

Material examined: PAKISTAN: Punjab Province: **Collections 1-8: District Gujranwala**, tehsil Gujranwala. **Collection 1**: Mandiala Tegha, in pairs, among grass, along the roadside, 226 meters (744 ft.) a. s. l., 27th September 2021, S. Rukhsar (GM-100); **Collection 2**: Dharamkot, solitary, S. Rukhsar (GM-97); **Collection 3:** Feroz Road, Jabboke, in pair, on moist soil, 25th September 2021, S. Rukhsar (GM-88); **Collection 4:** Sialkot road, Nawan Pind, solitary, on heap of waste, S. Rukhsar (GM-84); **Collection 5:** Aroop, in pairs, among grass, along the roadside, S. Rukhsar (GM-81); **Collection 6:** Sangowali, solitary, among grass, 4th September 2021, S. Rukhsar (GM-55); **Collection 7:** Feroz Wala Road, from muddy soil, along the road, 21th August 2021, S. Rukhsar (GM-6); **Collection 8:** Town Qila Didar Singh, growing between leaf litters near *Acacia nilotica* (L.) Willd. ex Delile, solitary, on the moist soil, 18th August 2021, S. Saeed, (GC-20); **Collection 9: District Kasur**, Green Valley near Kasur Bypass, solitary, growing on ground, terrestrial, at 218 m a. s. l., 30th Sep. 2021, M. Waseem, (MW-13); **Collection 10: District Khanewal**, Chak-15/v, in groups or clusters, on moist ground, among grasses, under *Acacia nilotica*, 24th July, 2020, M. Usman, (KU-60).

Discussion

Under-examined taxon was first reported by one of the Swedish mycologists, Magnus Fries as *Agaricus apalus.* Later on Arnolds gave the name *Conocybe apala*. White, non-striate pileus, extremely soft, crowded lamellae, and rapidly decomposing context are its defining characteristics. It is enormously delicate and ephemeral fungus (Amandeep *et al*., 2015).

Due to its conical to umbonate pileus, yellowish brown color, crowded gills, and related anatomical characters e.g., lecythiform cystidia and ellipsoidal spores' traits, *C. apala* can be confused with *C. pubescens* Sensu auct. brit., but latter has a striate pileus which is not the case of our species, in addition, former has white pubescent stem which is smooth in latter case.

C. apala closely resembles with *Bolbitius titubans* (Bull.) Fr., because of the shape and color of basidiospores but the spores in latter species are larger than basidiospores of the former. Morphologically, pileus of *C. apala* is different from that of *B. titubans*. which is orange to white compared to yellow to greenish yellow in latter (Amandeep *et al*., 2013).

C. apala can also be misidentified with *C. tenera* (Schaeff.) Fayod, as both have caps with a conical appearance in the initial stages of the development, cap surface is smooth in both the

species, and gills are pale ochre in premature specimens; however, the stipe of *C. apala* is white with a ceramic tint whereas stipe in *C. tenera* is white flushed with rusty brown and finely granular. Radial wrinkles, sometimes, appear on the cap surface of *C. apala* which distinguishes it from *C. tenera* (Hausknecht and Krisai-Greilhuber, 2006; Amandeep *et al*., 2015).

Based on detailed morpho-anatomical comparison, all the ten collections were found belonging to one taxon and later identified as *C. apala.* A comparison of *C. apala* specimens collected from three different districts (Gujranwala (2/8 collections compared), Kasur and Khanewal) in this study is given in Table 1.

Comments. One of the most widespread Agaric mushrooms is the *Conocybe apala*. The fruiting bodies grow abundantly during the rainy seasons, when there is a lot of moisture and dew in the air. Appearance of these specimens can be seen in spring and autumn. This saprobic mushroom exhibits habitat diversity, therefore can be found in grassland, parks, golf courses, on leaf litter, wooden substrates, and animal droppings (Amandeep *et al*., 2015). *C. apala* is considered inedible and it contains a class of toxins known as phallotoxins (Hallen *et al*., 2003).

Conclusion

It is concluded from this investigation that plains of Punjab province of Pakistan are rich in diversity of gilled mushrooms and this study yielded a new record of Agaric from Pakistan.

Conflict of interest

The authors declare that there is no conflict of interest related to this document.

References

Ahmad, S., S.H. Iqbal and A.N. Khalid. 1997. Fungi of Pakistan. *Sultan Ahmad Mycological Society of Pakistan, Lahore*, 248 pp.

Akçay, M. E., U.Z.U.N. Yusuf and S. Kesici. 2018. *Conocybe anthracophila*, A new record for the Turkish Mycobiota. *Ant. J. Bot*., 2(2): 84-87.

Amandeep, K., N.S. Atri and K. Munruchi. 2013. Diversity of species of the genus *Bolbitius* (Bolbitiaceae, Agaricales) collected on dung from Punjab, India. *Mycosphere*, 4(6): 1053-1064.

Amandeep, K., N.S. Atri and K. Munruchi. 2015. Diversity of species of the genus *Conocybe* (Bolbitiaceae, Agaricales) collected on dung from Punjab, India. *Mycosphere*, 6(1):19-42.

Arnolds, E. 2005. *Bolbitius*. W: Noordeloos ME, Kuyper TW, Vellinga EC (red.). Flora Agaricina Neerlandica. Critical monographs on families of agarics and boleti occurring in the Netherlands. *Taylor & Francis Group*, 112: 119.

Atri, N.S. 2005. Wild mushrooms-collection and identification. *Fronti. Mush. Biotech.*, 9-26.

Benedict, R.G., L.R. Brady, A.H. Smith and V.E. Tyler. 1962. Occurrence of psilocybin and psilocin in certain *Conocybe* and *Psilocybe* species. *Lloydia*, 25 (3): 156-159.

Benedict, R.G., V.E. Tyler and R. Watling. 1967. Blueing in *Conocybe*, *Psilocybe* and *Stropharia* species and the detection of psilocybin. *Lloydia*, 30: 150-157.

Brady, L.R., R.G. Benedict, V.E. Tyler, D.E.J. Stuntz and M.H. Malone. 1975. Identification of *Conocybe filaris* as a toxic basidiomycete. *Lloydia*, 38: 172-173.

Çagli, G., A. Öztürk and M.Z. Koçak. 2019. Two new basidiomycete records for the Mycobiota of Turkey. *Ant. J. Bot*., 3(2): 40-43.

Demirak, S.M.S., I. Turkekul and H. Isik. 2021. *Conocybe romagnesii* and *Gerronema subclavatum* (Basidiomycota: Agaricales) in the Central Black Sea Region of Turkey. *Nor. J. Bot.*, 39(12): 34-55.

Enderle, M. 1994. Conocybe-Pholiotina studien V. *Z. Mykol.*, 60 (1): 35-48.

Enderle, M. and H.J. Hübner. 1999. Studies in *Conocvbe* and *Pholiotina* VIII. *Z. Mykol.*, 65: 3-22.

Halbwachs, H. and C. Bässler. 2020. No bull: Dung-dwelling mushrooms show reproductive trait syndromes different from their non-coprophilous allies. *Mycol. Prog.*, 19(8): 817-824.

Hallen, H.E., R. Watling and G.C. Adams. 2003. Taxonomy and toxicity of *Conocybe lactea* and related species. *Mycol. Rec.*, 107 (8): 969-979.

Hausknecht, A. 2009. A *monograph of the genera Conocybe Fayod and Pholiotina Fayod in Europe*. Candusso, Alassio, 968pp.

Hausknecht, A. and I. Krisai-Greilhuber. 2006. Infrageneric division of the genus *Conocybe*-a classical approach. *Osterr. Z. Pilzkd.*, 15: 187-212.

Hausknecht, A.N.T.O.N. 1998. *Conocybe spinulosa*, a new species of *Conocybe* subg. *Ochromarasmius* from Tanzania. *Osterr. Z. Pilzkd.*, 7: 1-12.

Hausknecht, A., K. Kalamees, H. Knudsen and V. Mukhin. 2009. The genera *Conocybe* and *Pholiotina* (agaricomycotina, Bolbitiaceae) in temperate Asia. *Folia Cryptog. Estonica*., 45: 23-47.

Horak, E. and A. Hausknecht. 2002. Notes on extra-European taxa of Bolbitiaceae (Agaricales, Basidiomycota). *Osterr. Z. Pilzkd.*, 11: 213-264.

Izhar, A., H. Bashir and A.N. Khalid. 2019. A new species of *Conocybe* (Bolbitaceae) from Punjab, Pakistan. *Phytotaxa*, 402(5): 251-258.

Latha, K.D., S. Nanu, S.A Sharafudheen and P. Manimohan. 2018. Two new species of *Gerronema* (Agaricales, Basidiomycota) from Kerala State, India. *Phytotaxa*, 364(1): 81-91.

Liu, J. and T. Bau. 2018. New species and new records in the genus *Conocybe* (Bolbitaceae) from China. *Phytotaxa*, 357 (4): 261-274.

Malysheva, E.F. 2017. Five new species of *Conocybe* (Agaricomycetes, Bolbitiaceae) from Russia. *Mycol. Prog.*, 16(6): 625-636.

Malysheva, E.F., V.F Malysheva and T.Y. Svetasheva. 2015. Molecular phylogeny and taxonomic revision of the genus *Bolbitius* (Bolbitiaceae, Agaricales) in Russia. *Mycol. Prog.*, 14(8): 1-14.

Manimohan, P., K.A. Thomas and V.S. Nisha. 2007. Agarics on elephant dung in Kerala State, India. *Mycotaxon*, 99(1): 147-158.

Melo, R.F.R., R.D.S. Chikowski, AN. Miller and L.C. Maia. 2016. Coprophilous Agaricales (Agaricomycetes, Basidiomycota) from Brazil. *Phytotaxa*, 266(1): 1-14.

Pegler, D.N. 1977. A preliminary agaric flora of East Africa. *H. M. S. O.*, 6: 1-615.

Pegler, D.N. 1983. *Agaric flora of the Lesser Antilles*. Kew Bulletin Add. London. pp.668.

Pegler, D.N. 1986. Agaric flora of Sri lanka. *Kew Bul.l Addit. Ser.*, 12: 1-519.

Razaq, A., A. Nazir and A. Ishaq. 2022. Molecular and Micrographic Study Of *Chlorophyllum hortense*, ussing ITS-rDNA Marker and Scanning Electron Microscopy (SEM) Technique. *J. Plantarum*, 4(1): 48-57.

Singer, R. 1986. *The Agaricales in modern taxonomy*. 4th edn Koeltz Scientific Books. Koenigstein, Germany. 981.

Thomas, K.A., A. Hausknecht and P. Manimohan. 2001. Bolbitiaceae of Kerala State, India: New species and new and noteworthy records. *Osterr. Z. Pilzkd.*, 10: 87-114.

Watling, R. 1971. Observations on the Bolbitiaceae—IV. Developmental studies on *Conocybe* with particular reference to the annulate species. *Mol. Phylogeny Evol. Fungi.* 6(2): 281-289.

Watling, R. and A. Hausknecht. 1997. *Conocybe anthuriae*: a new volvate species from Mauritius (Africa). *Osterr. Z. Pilzkd.*, 6:55-59.

Fig. 1. A–E. Morphology of basidiomata of *Conocybe apala* (GM-100). A. Upper side of pileus. **B.** Underside of pileus showing lamellae. **C & D.** Different views of basidiomata. **E.** Stipe. **Scale bars: A & B=** 0.5, **C & E=** 1.0 cm, **D=** 0.8 cm.

Fig. 2. A-N. Different Collections of *Conocybe apala*. A. GM-6, **B.** GM-55, **C, L, M.** GC-20, **D, F**. GM-81, **E, G.** GM-84, **H.** GM-88, **I, J.** KU-60, **K, N.** MW-13. **Scale bars: A=** 2.5 cm, **B, F, G, H, M=** 1.0 cm, **C=** 2.5 cm, **D=** 4 cm, **E, K=** 1.7 cm, **I=** 1.3 cm, **J=** 2.4 cm, **L, N=** 0.8 cm.

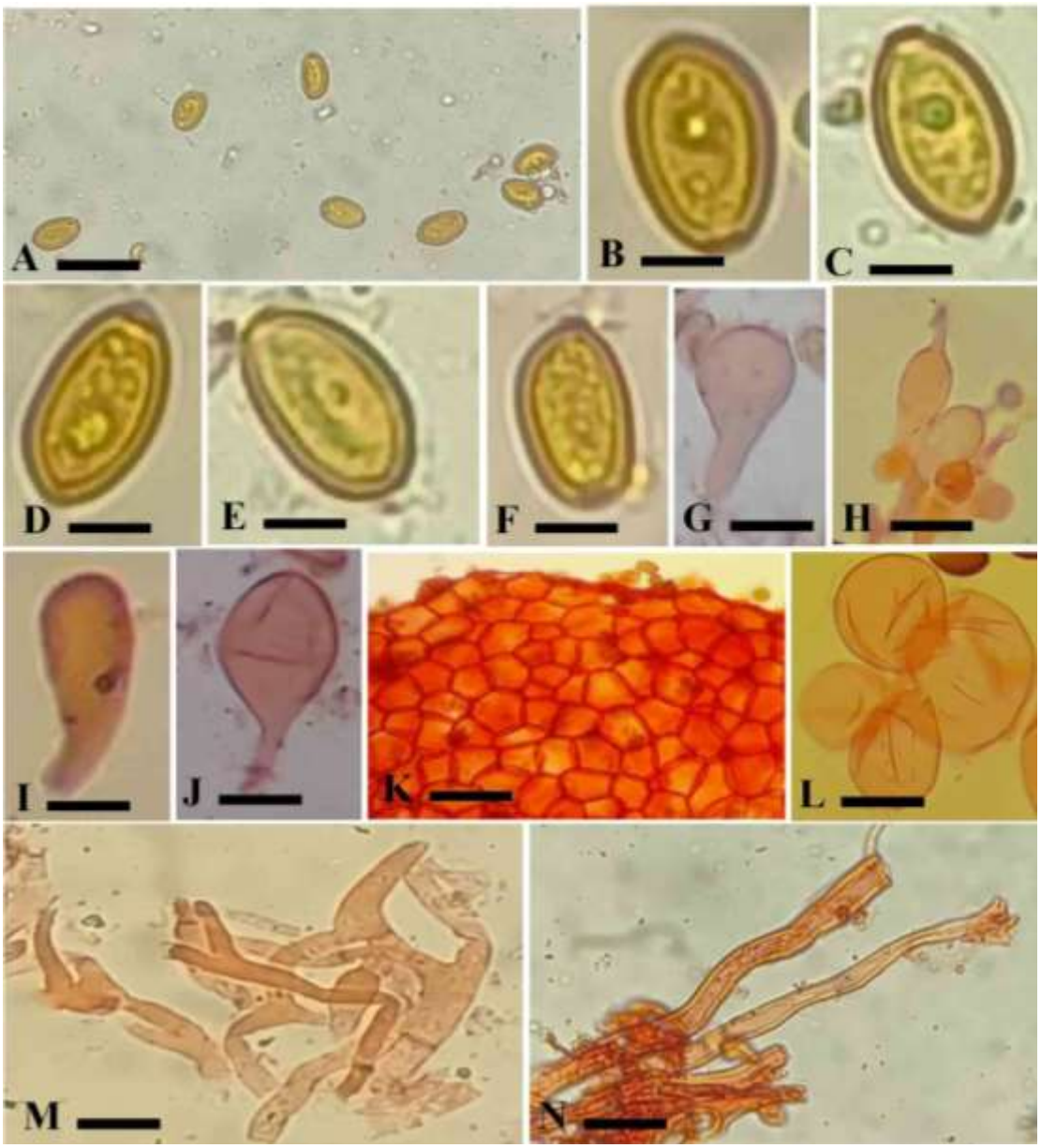

Fig. 3. A–N. Light micrographs of microscopic features of *Conocybe apala* (GM-100). A–F. Basidiospores. **G.** Basidia. **H–J.** Cheilocystidia. **K & L.** Pileipellis elements. **M.** Pileipellis hyphae. **N.** Stipitipellis. **Scale bars: A=** 20 µm, **B=** 3 µm, **C=** 3.5 µm, **D=** 4.0 µm, **E=** 3.0 µm, **F=** 5.0 µm, **G=** 11.5 µm, **H=** 7.2 µm, **I=** 6.5 µm, **J=** 7.0 µm, **K=** 42.0 µm, **L=** 13.0 µm, **M=** 7 µm, **N=** 12.2 µm.

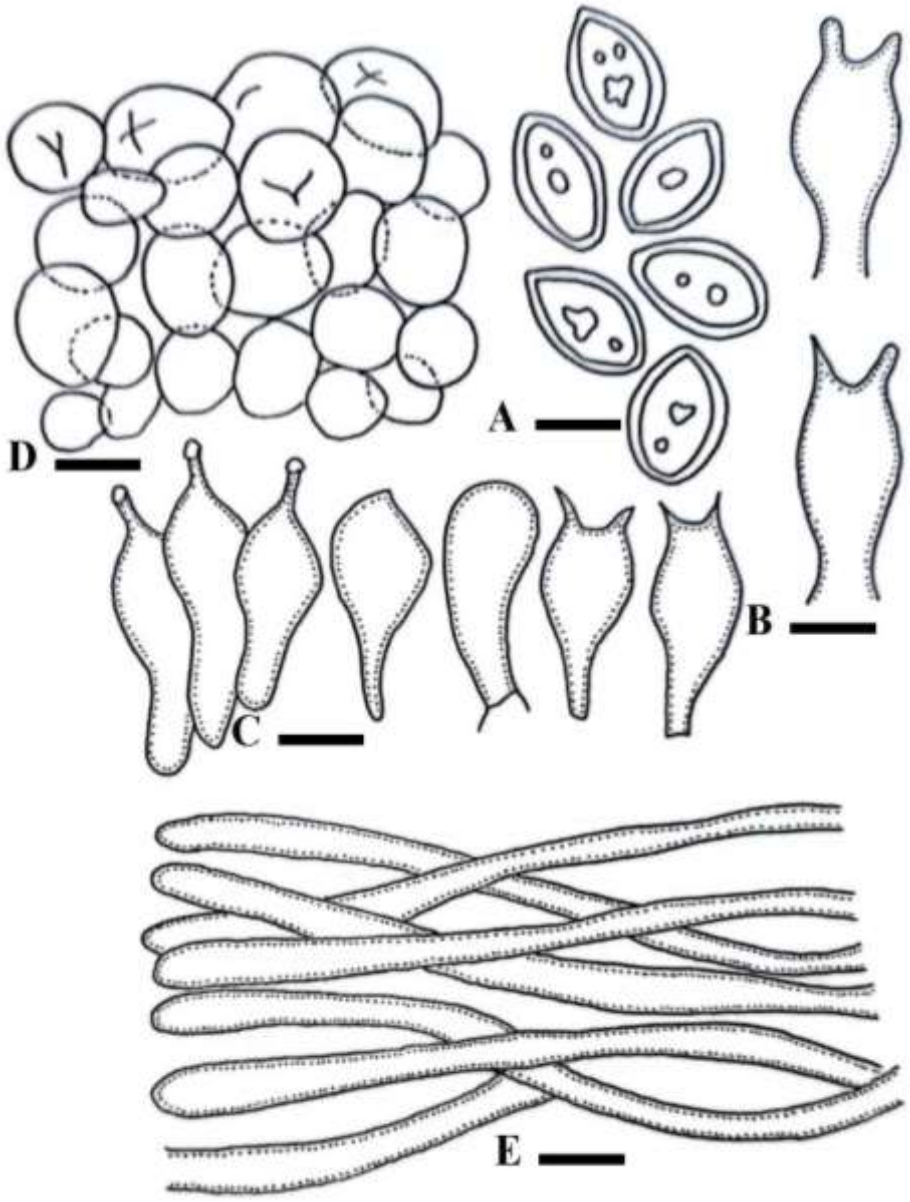

Fig. 4. A–E. Illustrations of microscopic features of *Conocybe apala* (GM-100). A. Basidiospores. **B.** Basidia. **C.** Cheilocystidia. **D.** Pileipellis elements. **E.** Pileipellis. **Scale bars: A=** 8.2 µm, **B=** 13 µm, **C=** 7.2 µm, **D=** 19.5 µm, **E=** 8.5 µm.

Table 1: A comparison of specimens of *Conocybe apala* collected from different areas of Punjab, Pakistan

C. apala	**Locality/District**	**Habitat**	**Pileus**			**Color of lamellae**	**Stipe length**	**Basidiospores**		**Basidia**		**Cheilocystidia**	
			Shape	**Size**	**Color**			**Shape**	**Size**	**Shape**	**Size**	**Shape**	**Size**
KU-60	Khanewal	on wet soil, under *Acacia nilotica*	conico-campanulate to conico-convex	2.2-3.6 × 2.8-3.5 cm	whitish to creamy buff	pale brown to cinnamon brown	7.8-9.4 × 0.4 cm	oblong	8.5-14.2 × 7.0-8.5 µm	clavate or oblong	22.7-28.5 × 14.0-17.0 µm	Lecythiform with sub-globose head	17.2-25 × 11-20.3 µm
GM-100	Gujranawala (Mandiala Tegha)	among grass, on moist soil, & heap of waste	campanulate	2.5-4 × 2-3 cm	greyish to slightly orange from center	pale brown to cinnamon brown	7-8.5 × 0.3-0.5 cm	oblong	11-17 × 5-8 µm	clavate or oblong	28-42 × 7-20 µm	Lecythiform with sub-globose head	17.0-28.2 × 12.0-22.7 µm
GC-20	Gujranawala (Qila Didar Singh)	on leaf litter, on moist soil, under *Acacia nilotica*,	broadly conical to campanulate	2.4-3.8 × 2.5-3.1 cm	whitish to creamy buff	light brown to cinnamon brown	7.8-8.9 × 0.2-0.6 cm	oblong	11.5-16 × 6.0-8.2	broadly oblong	28.5-34 × 11.5-19 µm	Lecythiform shape	25.6-31.4 × 11.4-15.7 µm
MW-13	Kasur	on ground	conical	3.2 × 1.9 cm	white to cream	pale brown to brown	6.7 × 0.2 cm	oblong to ellipsoid	12-17 × 5–7 µm	broadly clavate	34–48 × 10–14 µm	Lecythiform	38–41 × 17–20 µm