Acta Botanica Brasilica, 2022, 36: e2021abb0382
doi: 10.1590/0102-33062021abb0382

Original article

Lepiota brunneogranulosa (Agaricaceae): a new species from Punjab, Pakistan, based on integrated taxonomy

Muhammad Asif[1*], Aiman Izhar[1], Muhammad Haqnawaz[1], Abdul Rehman Niazi[1] and Abdul Nasir Khalid[1]

Received: December 24, 2021
Accepted: August 29, 2022

ABSTRACT

During our surveys of mushrooms in 2019 and 2020, an interesting species of the genus *Lepiota* was collected from three different areas of Punjab (Bahawalnagar, Sheikhupura, and Muzaffarghar), Pakistan. These areas vary in their altitude and somewhat climatic conditions, nevertheless, morpho-anatomical comparisons and molecular analyses proved that these collections represented the same taxon, described here as *Lepiota brunneogranulosa*. This new species is characterized by a pileus with a yellowish-brown central disc, with brown colored granules on a very pale brown background, dextrinoid, ellipsoid basidiospores, frequently bi-sporic basidia, and narrowly clavate cheilocystidia. Because of the hymeniform pileus covering, and the placement in the phylogeny, based on Internal Transcribed Spacer (nrITS) and Larger Subunit (LSU), it belongs to the *Lepiota* sect. *Lilaceae*.

Keywords: Bahawalnagar, Lepiotaceous fungi, litter-inhabiting, Phylogeny, taxonomy

Introduction

Species of the genus *Lepiota* (Pers.) Gray (1821: 601) are saprobes in nature (Singer 1986; Vellinga 2004) and globally distributed from tropical to temperate areas and are infrequent in deserts and arctic-alpine habitats (Vellinga 2004) with more than 500 species worldwide (Singer 1986; Kirk *et al.* 2008; Razaq *et al.* 2012; Nawaz *et al.* 2013; Qasim *et al.* 2015; Sysouphanthong *et. al* 2016; Bashir *et al.* 2020; Niazi *et al.* 2021), including more than 30 species reported from Pakistan (Ahmad *et al.* 1997; Kirk *et al.* 2008; Kumar & Manimohan 2009; Liang & Yang 2011; Nawaz *et al.* 2013; Razaq *et al.* 2012; 2013; 2014; Qasim *et al.* 2015; Qasim *et al.* 2016; Bashir *et al.* 2020; Niazi *et al.* 2021). The genus *Lepiota* is characterized by its white to pale, mostly dextrinoid basidiospores that have different shapes *i.e.* ellipsoid to spurred, fusiform or penguin-shaped, various pileus coverings, ranging from hymeniderm, cutis, trichoderm, epithelium, a regular hymenophoral trama and usually the presence of clamp connections (Candusso & Lanzoni 1990; Vellinga 2003).

1 Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, 54590, Lahore, Pakistan

*Corresponding author: asifgondal101@gmail.com

Pileus covering and basidiospore shape are the key characteristics that divide the genus *Lepiota* into several sections (Vellinga 2001a). Species within the genus *Lepiota* show quite close affinities on morphological analysis and have further been divided into several sections based on molecular data. *Lepiota* species have been supported by molecular studies indicating that the genus is polyphyletic, and thorough work focused on multigene phylogenetic analyses has placed the members of the genus in the relevant sections. (Sysouphanthong *et al.* 2011). However, there are still a lot of species that need to be identified and described.

Presently, there are six sections within the genus *Lepiota* and each section has a unique structure of pileus covering and basidiospore shape, viz., (1) species with fusiform-amygdaliform spores and trichodermal pileus covering (sect. *Lepiota*), (2) species with subglobose to ellipsoid or spurred spores and hymenidermal pileus covering (sect. *Lilaceae* M. Bon), (3) species with ovoid to ellipsoid spores and cylindrical pileus covering elements without clamp-connections (sect. *Fuscovinaceae* (J. Lange) Kühner), (4) species with ovoid to ellipsoid spores and trichodermal pileus covering (sect. *Ovisporae* (J. Lange) Kühner), (5) species with spurred spores and trichodermal or cutis pileus covering (sect. *Stenosporae* (J. Lange) Kühner) and (6) species with ovoid to ellipsoid or cylindrical spores with a spurred base and epithelium pileus covering (elements agglutinated in chains) (sect. *Echinatae* Fay.) (Candusso & Lanzoni 1990; Bon 1993; Vellinga 2001c).

Lepiota sect. *Lilaceae* is distinguished by a hymenidermal pileus covering which is made up of firmly packed clavate to narrowly clavate elements, and by subglobose to ellipsoid or spurred basidiospores (Bon 1981; Vellinga 2001a). Section *Lilaceae* is dissimilar from the other five sections of *Lepiota* based on morphological and molecular evidence (Vellinga 2001a; Vellinga 2003). However, some species, despite producing spurred spores like members of the sect. *Liliaceae*, were placed in some other sections of the genus in having close phylogenetic relationships with those sections (Candusso & Lanzoni 1990; Bon 1981; Horak 1981).

Morphological differences between *Lepiota* species are often quite subtle (Vellinga & Huijser 1998). Fortunately, the separation of different species has become more precise and exact based on nrITS phylogenetic analyses (Vellinga 2003).

Lepiotaceous fungi are highly diverse group, especially in tropical and subtropical areas, and a huge amount of work has been done on species from different sections of the genus, both based on morphology and phylogeny (Vizzini *et al.* 2014; Justo *et al.* 2015; Hosen *et al.* 2016; Sysouphanthong *et al.* 2020). They are found in many localities of Punjab Pakistan. Here, a new species of the genus *Lepiota* sect. *Lilaceae,* collected in three districts (Bahawalnagar, Sheikhupura, and Muzaffarghar) of Punjab, Pakistan, is presented with a description, illustrations, and phylogenetic analyses of its nrITS and LSU sequences.

Materials and methods

Locality description

The type specimen was collected from district Bahawalnagar, Punjab, Pakistan during the rainy season of 2019. Geographically, District Bahawalnagar is situated in eastern Punjab (Coordinates: 30°33′02″ N, 73°23′26″ E, 163 m) on the bank of Satluj River. The temperature of the district varies from a minimum of 11 °C to a maximum of 50 °C and the average annual precipitation is 119 mm (Ahmed *et al.* 2014a; 2014b). The second collection was collected from district Sheikhupura, in central Punjab (Coordinates: 31°42'47" N, 73°58' 41" E). The climatic conditions of the district remain moist sub-humid with annual rainfall from 250 to 500 mm which reaches a maximum of 635 mm (Nawaz *et al*. 2017). Another collection was made from District Muzaffargarh, in south-western Punjab, (Coordinates: 30°4′10″ N, 71°11′39″ E, 65 m), which lies in a sub-humid region with 127-150 mm average annual rainfall (Nickson *et al.* 2005; Akram *et al.* 2014; Zamir & Kazmi 2014; Mahmood *et al.* 2019).

Morphological Protocols

Basidiomata were collected, photographed, and the main fleeting features and superficial characters along with habitat and geographical data were noted in the field. Basidiomata were described using the terminology of Vellinga (2001b). Munsell's soil color chart (Munsell 1975) was used to describe the colors. The collected samples were brought to the laboratory and dried at 40-50 °C using a fan heater. The dried specimens (Holotype) were deposited in LAH Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan, and holotype (=isotype) was deposited in Islamabad Herbarium under herbarium voucher no. ISL-F02.

For anatomical analysis, dried tissues were rehydrated in 5 % KOH, stained with 1 % Congo Red; Melzer's reagent was used for reactions of the spore walls. The microscopic features such as shape and size of basidiospores, cheilocystidia, basidia, structure, and elements of pileus covering and stipe covering were observed using a light microscope (CXRII, Labomed Labo America Inc., Fremont, CA, USA) with an HDCE-X5 microscopic camera under 40X and oil immersion 100X magnification. From each collection, a minimum of 25 measurements were taken for each character. Abbreviation: Dimension a–b × c–d = minimum and maximum values of length × width, avw = average width, $L_m \times W_m$ = mean spore length × mean spore width, spore quotient Q = length/width, Qm = mean quotient of all basidiospores. The notation 'n/b/p' indicates 'n' basidiospores measured from 'b' basidiomata from 'p' collections (Bas 1969; Yu *et al.* 2020).

Molecular Protocols

DNA extraction followed the 2 % CTAB protocol (Bruns 1995) using dried specimens. DNA was amplified using a combination of primers (*i.e.* ITS1F/ITS4) for ITS and LR0R/LR5 for 28S (Vilgalys & Hester 1990; Gardes & Bruns 1993; White *et al.* 1990). PCR was done in 25 µl reaction volume following Gardes & Bruns (1993). The PCR products were sequenced by a commercial laboratory and newly generated sequences in this study were deposited in GenBank under accession numbers nrITS OL331703 - OL331705 and LSU OL331514 - OL331516.

Phylogenetic analyses

The three obtained sequences from both ITS and LSU regions were prepared using BioEdit (Hall 1999). The final dataset of ITS sequences was retrieved from GenBank based on maximum percentage identity from BLAST results and from phylogenetic studies on *Lepiota* (Hosen *et al.* 2016; Sysouphanthong *et al.* 2020) it consisted of 65 ingroup taxa and *Macrolepiota dolichaula* Pegler & R.W. Rayner (1969: 365) (JQ928939) as an outgroup. The LSU final dataset retrieved from GenBank comprised 26 sequences including *Macrolepiota dolichaula* (KY418836) as an outgroup taxon. We used the Basic Local Alignment Search Tool (BLAST) (Altschul *et al.* 1990) against the GenBank database to find taxa with closely allied sequences, which were then retrieved for addition in the phylogenetic analyses dataset. All the sequences were aligned using the Multiple Sequence Alignment search tool (https://www.ebi.ac.uk/Tools/msa/muscle/) (Edgar 2004) and then adjusted manually in BioEdit (Hall 1999). Final alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession numbers http://purl.org/phylo/treebase/phylows/study/TB2:S28978 and http://purl.org/phylo/treebase/phylows/study/TB2:S28980. Maximum Likelihood (ML) analysis was done using RAxML-HPC2 v. 8.2.12 (Stamatakis 2014) as applied on the CIPRES portal v 3.1 (Miller *et al.* 2010) and with 1000 rapid bootstrap iterations for both genes.

Results

Phylogenetic analyses of ITS dataset

The fragment size of the target region was 660 bp long. In BLAST results, the ITS sequences of *Lepiota brunneogranulosa* showed 86 % maximum similarity with *Lepiota* sp. (AF391052) from the USA. In this analysis, *L. brunneogranulosa* gets clustered within the *L.* sect. *Lilaceae* close to *L. lilacea* (1892: 3) (GQ203822 & AY176379) reported from California, USA and *L. bengalensis* (2016: 188) (KU563149 & KU563148) reported from Bangladesh (Fig. 1).

Phylogenetic analyses of LSU dataset

The fragment size of the large subunit target region was 926 bp long. The ITS sequences of *Lepiota brunneogranulosa* showed 97 % maximum similarity with *Lepiota* sp. (OL652983), an unpublished species from Australia. In this analysis, *L. scaberula* Vellinga (2001: 290) (MK278271) reported from the USA is closely related to our new species (Fig. 2).

Taxonomy

Lepiota brunneogranulosa M. Asif, A. Izhar, Haqnawaz, Niazi & Khalid *sp. nov.*

Figs. 3A-D, 4A-E

MycoBank No. MB841888

Etymology: brunneogranulosa (Latin), referring to the brown-colored granules on the pileus surface.

Diagnosis: This species is characterized by brown granules on pileus, stipe turning yellowish on handling, narrowly clavate cheilocystidia and hymeniderm pileus covering with obovoid to utriform terminal cells.

Type: Pakistan. Punjab: Haroonabaad, District Bahawalnagar, (Coordinates: 30°33′02″ N, 73°23′26″ E; 163 m above sea level (*a.s.l.)*), Scattered or in groups, on nutrient-rich loamy soil, July 31, 2019, *Muhammad Asif BWN 23* (Holotype-LAH36803)**.** GenBank: ITS = OL331703; 28S = OL331514. (=Isotype ISL-F02).

Description: **Pileus** 1.7-2.6 cm, plano-convex at younger stage, later become plane at maturity, yellowish-brown (10YR5/6) central disc with brown (7.5YR4/3) granules on very pale brown (10YR8/4) surface, denser around the central disc and becoming sparse towards margins, showing the pale brown surface; dull and dry; margin undulating and dentate. **Lamellae** free, sub-distant, broad, light brown (6YR7/1), wavy with eroded edges, a single tier of lamellulae, concolorous. **Stipe** 2.8-3.5 × 0.2-0.5 cm light brown (6YR7/1), becoming yellowish upon handling, central, equal, with shiny surface, concolorous fibrils at the apex of stipe. **Annulus** sub-peronate, white (2.5YR5/12), single-edged, upturned, attached at the upper part of the stipe, disappearing at maturity. **Volva** absent. **Smell** acrid. **Taste** mild

Basidiospores (100/4/4) (4.5-) 4.6-5.2 × 3.2-3.8 (-3.9) µm, avl × avw = 4.9 × 3.5 µm, Q = 1.4 - 1.7, Qm = 1.5, thin-walled, pale yellow in KOH, slightly brownish in Melzer's reagent, but not so strong, ellipsoid, oblong in side view, rounded triangular in frontal view, non-guttulate, smooth with a prominent apiculus. **Basidia** 18-22 × 6.8-9 µm, avl × avw = 20.19 × 7.69 µm, Q = 2.62, thick-walled, mostly clavate, rarely narrowly clavate, frequently bisporic, rarely tri and tetra sporic, hyaline in KOH, non-guttulate. Lamella edge sterile, cheilocystidia crowded. **Cheilocystidia** 17-23 × 6-7.5 µm, avl × avw = 19.6 × 7.02 µm, Q = 2.8, thick-walled, narrowly clavate, hyaline in KOH, non-guttulate. **Pleurocystidia** absent. **Pileus covering** hymeniderm**,**

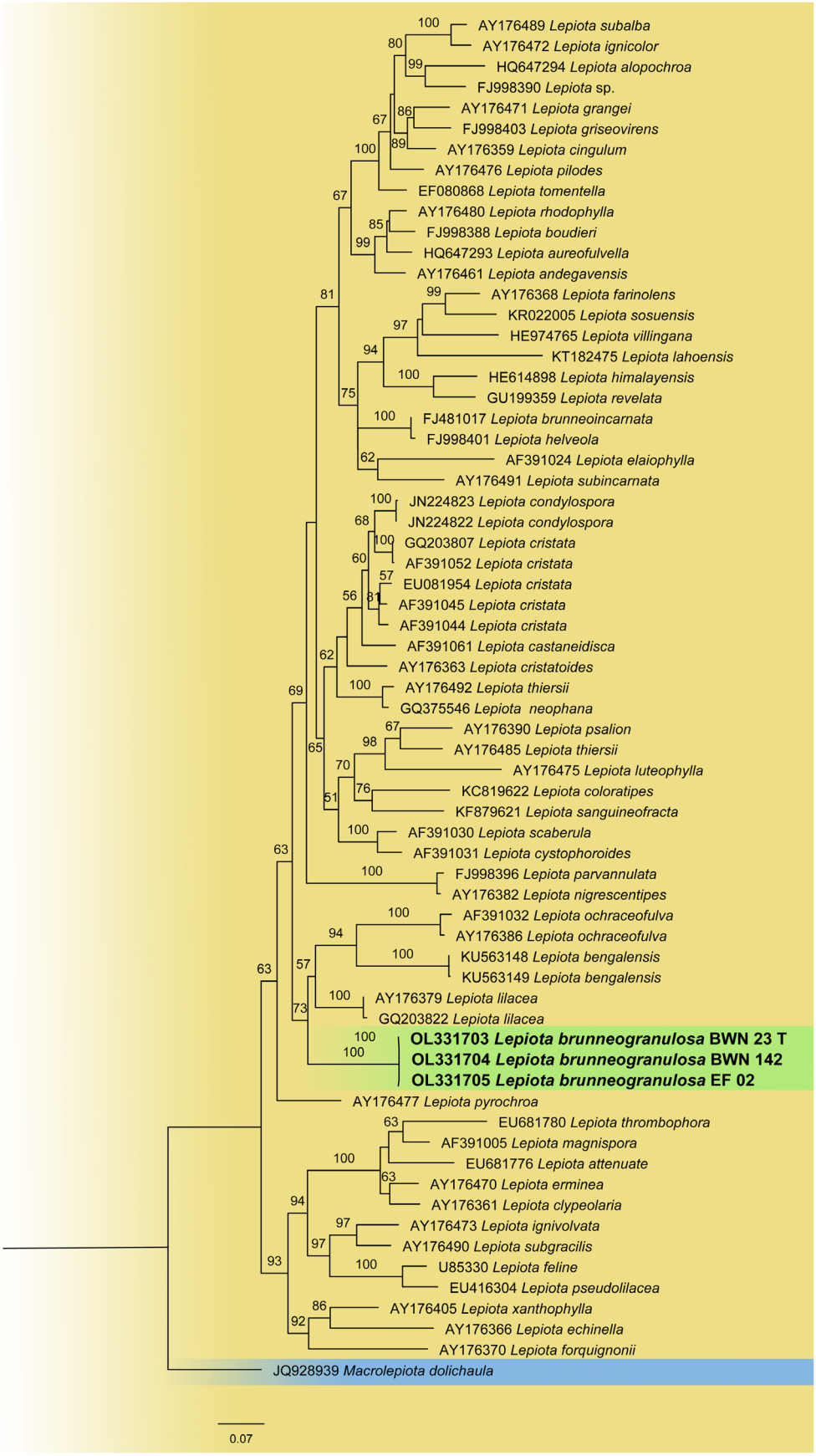

Figure 1. Molecular phylogenetic placement of *Lepiota brunneogranulosa* based on maximum likelihood (ML) method of ITS sequences.

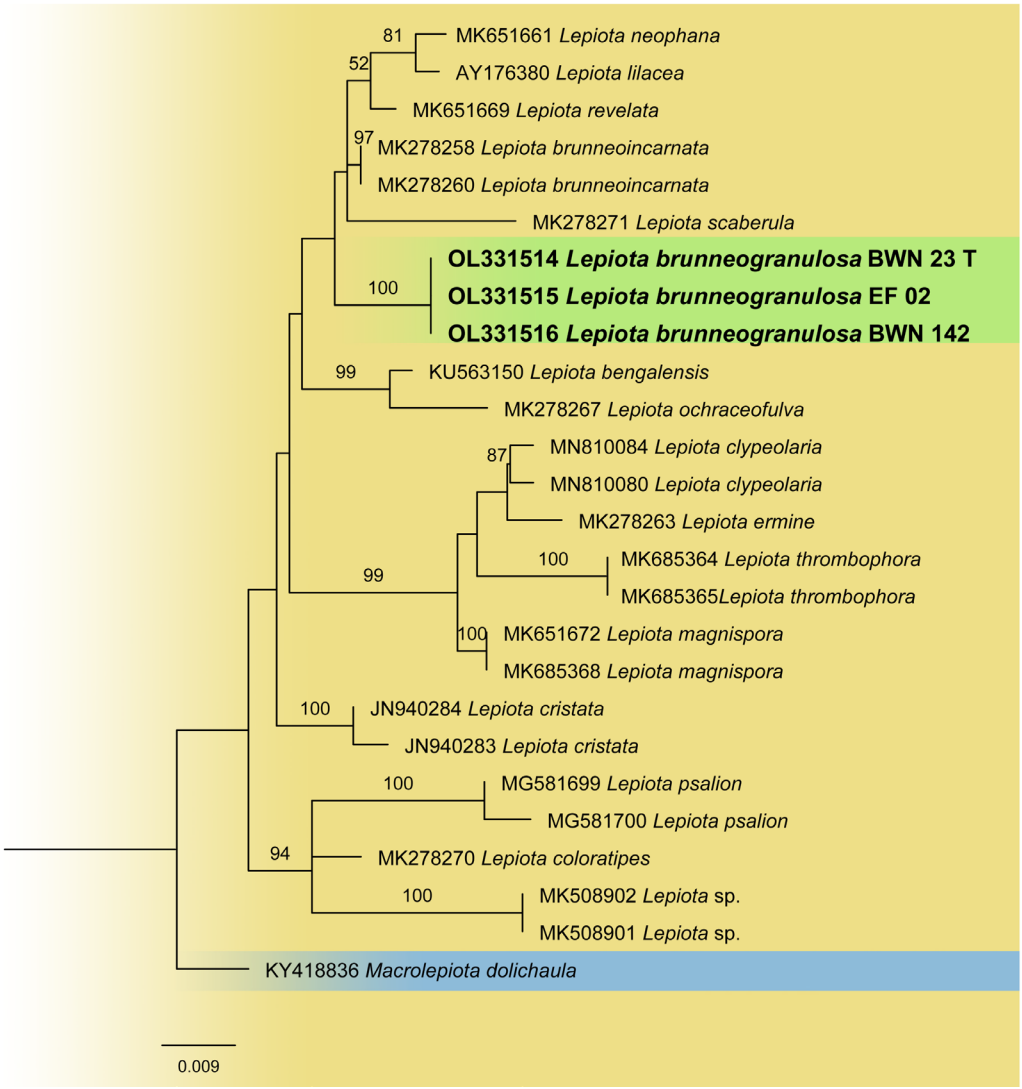

Figure 2. Molecular phylogenetic placement of *Lepiota brunneogranulosa* based on maximum likelihood (ML) method of LSU sequences.

thick-walled, hyaline in KOH, septate, irregularly arranged with obovoid, clavate to broadly clavate or utriform with median constriction terminal cells 25-47 × 17-27 µm, hyphae 8.7-6.3 µm, avl × avw = 19.3 × 11.5 µm. **Stipitipellis** a cutis, hyphae 6.6 – 13.2 µm, avw = 8.4 µm, thick-walled, hyaline in KOH, septate, parallel arrangement, long and narrow. **Caulocystidia** absent. **Clamp connections** are present in all tissues.

Habitat and Ecology: Scattered or in groups, on nutrient-rich loamy soil, under *Eucalyptus camaldulensis* Dehnh., Aug to Sep.

Geographical distribution range: Known only from Punjab, Pakistan.

Additional specimens examined: PAKISTAN. Punjab: Haroonabad, District Bahawalnagar, at 163 m *a.s.l*, September 07, 2020, *Muhammad Asif BWN 142* (LAH36804). GenBank: ITS = OL331704; 28S = OL331516. Pakistan-Punjab: District Sheikhupura, (Coordinates: 31° 71′67″N, 73°98 50″E; 236 m *a.s.l*) scattered on loamy soil, September 20, 2019, *Aiman Izhar SKP 1211* (LAH36974). Pakistan-Punjab: Easan Wala Forest, District Muzaffargarh, (Coordinates: 30°4′10″N, 71°11′39″E; 123 m *a.s.l*) August 29, 2020, *Muhammad Haqnawaz EF 02*, (LAH36954). GenBank: ITS = OL331705; 28S = OL331515.

Discussion

Lepiota brunneogranulosa is characterized by pileus with brown granules on a very pale brown background, presence of annulus on stipe, becoming yellowish on handling, narrowly clavate cheilocystidia, ellipsoid to broadly basidiospores, and obvoid, clavate to broadly clavate or utriform terminal elements in pileus covering (Tab. 1).

Lepiota lilacea (GQ203822 & AY176379) and *L. bengalensis* (KU563149 & KU563148) are found to be

close relatives of our newly described taxon in ITS based phylogenetic analysis (Fig. 1). But both species can be differentiated from our new species morpho-anatomically.

Lepiota lilacea is widely reported around temperate areas. It was originally described and widely reported in Europe and is also present in North America. It differs from *L. brunneogranulosa* macroscopically in having plano-convex to applanate pileus with dark violet central disc that has radially arranged scales, white lamellae with free margins, and microscopically in having ellipsoid to oblong basidiospores with 5.2 × 3.1 µm average size (Tab. 1) (Vellinga 2001ab; Vellinga *et al.* 2011).

Lepiota bengalensis reported from Bangladesh differs from *L. brunneogranulosa* in having pastel red to brownish red, conico-convex to hemispherical pileus with the dark ochraceous or reddish-brown central disc, and brownish red to reddish-orange appressed or radially arranged squamules on the pileus and no color change on touching (Hosen *et al.* 2016). Clamp connections are only present in pileus covering (Tab. 1) (Hosen *et al.* 2016).

In LSU based phylogenetic analysis (Fig. 2), *Lepiota scaberula*, a species originally reported from the California, USA, is the closest relative to *L. brunneogranulosa;* it differs from latter by its scurfy pileus surface with fibrillose v-shaped

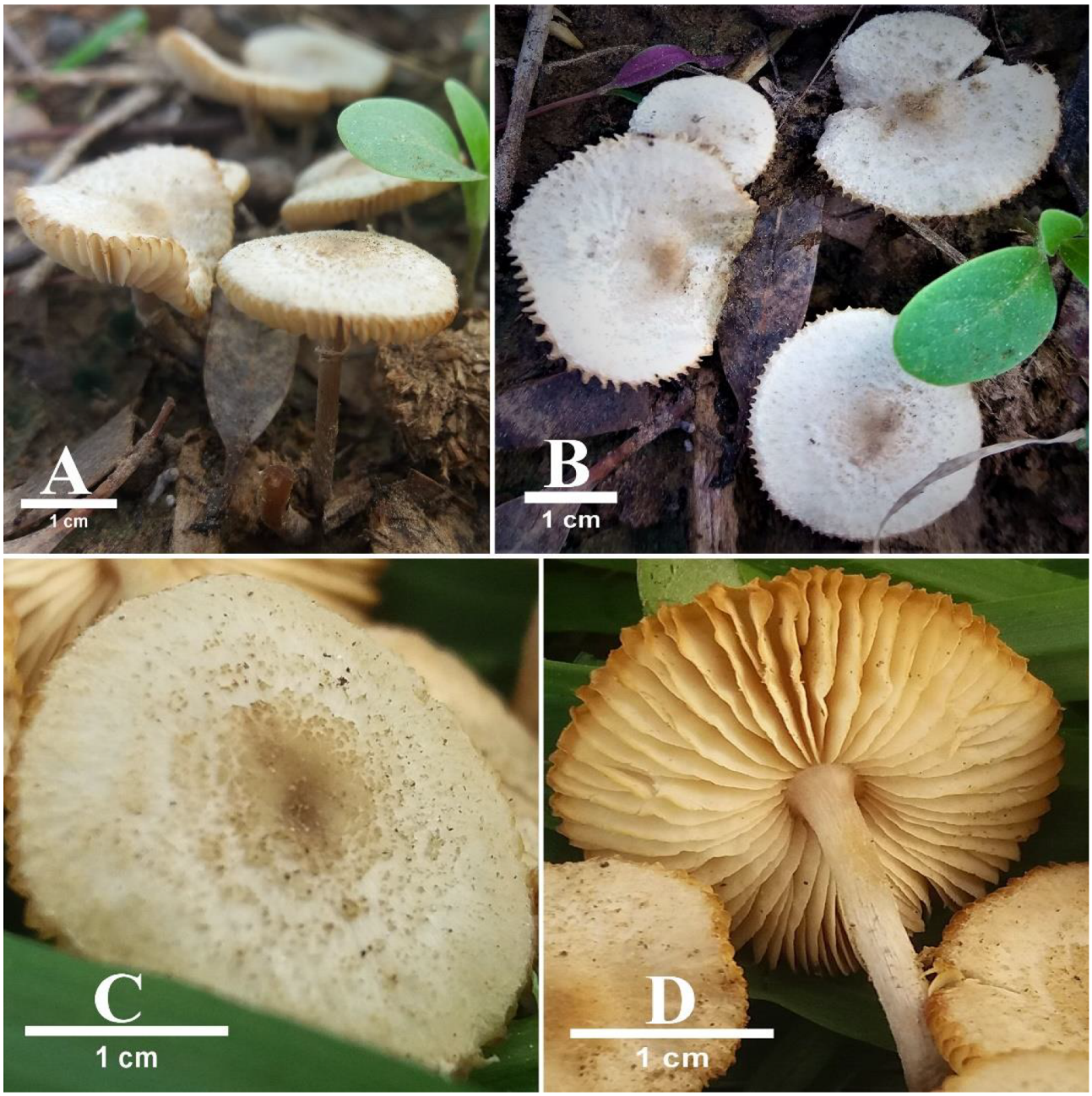

Figure 3. Basidiomata of *Lepiota brunneogranulosa* **(**LAH36803, Holotype) Photos by Muhammad Asif & Aiman Izhar.

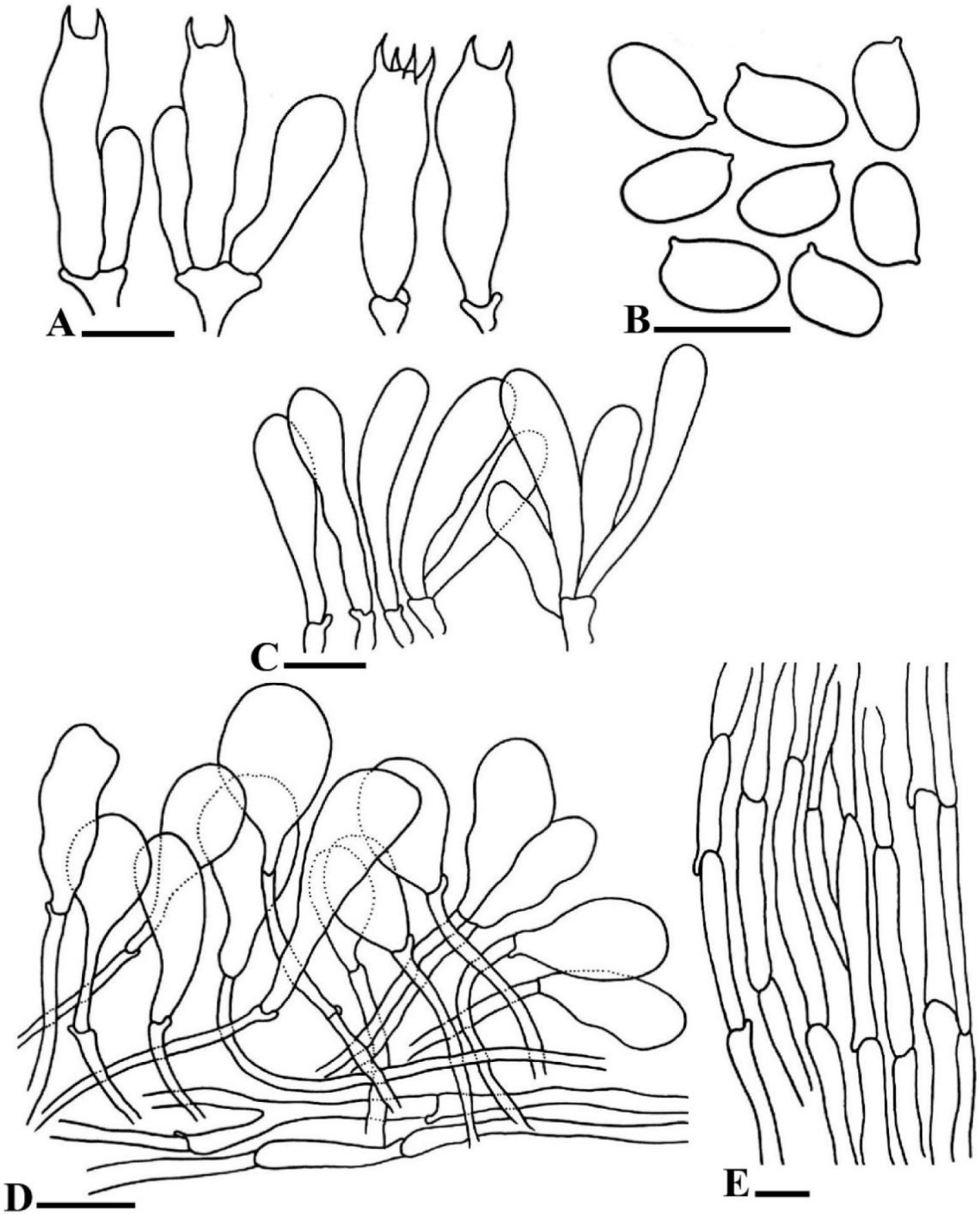

Figure 4. Microscopic characters of *Lepiota brunneogranulosa* (LAH36803, Holotype) **A.** Basidia, **B.** Basidiospores, **C.** Cheilocystidia, **D.** Pileus covering, **E.** Stipitipellis, Bars: A-E = 10 µm. Drawings by Aiman Izhar.

Table 1. Comparison of the diagnostic characteristics of *L. brunneogranulosa* with closely related species (Vellinga 2001b; 2001d; Hosen *et al.* 2016; Orton 1960).

Characters	*L. brunneogranulosa*	*L. lilaceae*	*L. bengalensis*	*L. scaberula*	*L. ochraceofulva*
Pileus	Plane, very pale brown	Violet, plano-convex to applanate,	Pastel red to brownish red, campanulate to conicoconvex, expanding to hemispherical to applanate with age	Paler around centre and whistish at margins, hemispherical to plano-convex	Orange brown to leather brown
Central disc	Yellowish-brown	Dark violet	Dark ochraceous or reddish brown	Cream to yellow-brownish	Reddish brown
Squamules	Brown granules	Violet, radially arranged appressed scales	Brownish red to reddish orange, appressed or radially arranged squamules	Irregularly fibrillose v-shaped squamules	Pileus broken into squamules
Lamellae	Sub-distant, light brown	Crowded, white	Distant to sub-crowded, pale yellow to yellowish white or light ochraceous-cream	Moderately crowded to moderately distant	Crowded
Basidiospores	Ellipsoid to broadly ellipsoid or oblong, av = 4.9 × 3.5 µm,	Ellipsoid to oblong, av = 5.2 × 3.1 µm	Ellipsoid to cylindrical, av = 5.6 × 3 µm	Ellipsoid to oblong, av = 5.7 × 3.6 µm	Broadly ellipsoid, av = 5.5 × 3.7 µm
Cheilocystidia	Narrowly clavate	Narrowly cylindrical, narrowly utriform	Fasciculate, clavate to narrowly clavate	Cylindrical and slightly widened at apex	Clavate
Pileus covering	Hymeniderm, irregularly arranged with obvoid, clavate to broadly clavate or utriform terminal cells	Hymeniderm made up of clavate to broadly clavate cells	Hymeniform, composed of clavate to broadly clavate or narrowly clavate cells	Cylindrical to slightly inflated hyphae	Hymeniform
Habitat	Scattered or in groups, on nutrient-rich loamy soil under Eucalyptus camaldulensis	Gregarious in small groups, on irrigated soil, on rich nitrophilous shrubs in woods under Sequoia sempervirens	Solitary, caespitose or gregarious in small groups on debris of Albizia and Eucalyptus	Gregarious, saprotrophic on soil in Sequoia sempervirens forests	On loamy soil, in gardens and parks

squammules, moderately crowded and pinkish lamellae, whitish-wooly stipe and non-dextrinoid basidiospores (Tab. 1) (Vellinga 2001d).

Lepiota ochraceofulva, which is originally reported from Europe, differs from *L. brunneogranulosa* by its crowded lamellae, long and broad stipe 52-70 × 6-9 mm with an almost clavate bulbous base, tinged ochraceous-cream color at stipe apex, and slightly wider basidiospores 5.5-7 × 3.5-4.5 µm (Tab. 1) (Orton 1960).

Previously, 32 species of *Lepiota* have been reported from Pakistan (Ahmad *et al.* 1997; Razaq *et al.* 2012; Nawaz *et al.* 2013; Qasim *et al.* 2015; 2016; Bashir *et al.* 2020; Niazi *et al.* 2021; Haqnawaz *et al.* 2022). Our current study, based on morphological and molecular data of the species collected from Punjab, Pakistan, indicated that this is a genuine member of the genus *Lepiota* and also a new taxon to science. So, future mycological expeditions are appreciated to explore more hidden species of the genus, their variabilities, and ecology from Pakistan.

Acknowledgments

We are sincerely thankful to Dr. Else C. Vellinga (University of California, Berkeley, USA) for her critical review and valuable comments and suggestions on an earlier version of this manuscript which helped us to improve the article. We thank all the anonymous reviewers for their corrections and suggestions to improve this paper.

References

Ahmad S, Iqbal S, Khalid AN. 1997. Fungi of Pakistan. Lahore, Sultan Ahmad Mycological Society of Pakistan.

Ahmed N, Mahmood A, Tahir SS, *et al.* 2014a. Ethno-medicinal knowledge and relative importance of indigenous medicinal plants of Cholistan desert Punjab Province. Pakistan Journal of Ethno-pharmacology 155: 1263-1275.

Ahmed N, Mahmood A, Tahir SS, *et al.* 2014b. Relative importance of indigenous medicinal plants from Layyah district, Punjab Province. Pakistan Journal of Ethno-pharmacology 155: 509-523.

Akram Z, Hussain S, Mansoor M, *et al.* 2014. Soil fertility and salinity status of Muzaffargarh District, Punjab Pakistan. Universal Journal of Agriculture Research 2: 242-249.

Altschul SF, Gish W, Miller W, *et al.* 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410.

Bas C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. Persoonia 5: 96-97.

Bashir H, Usman M, Khalid AN. 2020. *Lepiota cholistanensis* a new species of Lepiota (Agaricaceae: Basidiomycota) from Cholistan desert, Pakistan. Phytotaxa 455: 267-276.

Bon M. 1981. Clé monographique des "Lépiotes" d'Europe. Documents Mycologiques 11: 1-77.

Bon M. 1993. Flore mycologique d'Europe 3. Les Lépiotes. Documents Mycologiques Mémoire hors série 3: 1-153.

Bruns TD. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil 170: 63-73.

Candusso M, Lanzoni G. 1990. *Fungi Europaei 4*: *Lepiota s.l.* Alassio, Giovanna Biella.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792-1797.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes-Application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113118.

Gray SF. 1821. A Natural Arrangement of British Plants. Vol. II According to Their Relations to Each Other as Pointed Out by Jussieu, De Candolle, Brown, *et al.*. Baldwin,Cradock, and Joy. pp. 601.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT/7. Nucleic Acids Symposium Series 41: 95-98.

Haqnawaz M, Niazi AR, Usman M, *et al.* 2022. *Lepiota sindhudeltana* sp. nov. (Agaricaceae; Basidiomycota) from Punjab, Pakistan. Phytotaxa 550: 253-262.

Horak E. 1981. On Australasian species of *Lepiota* S.F. Gray (Agaricales) with spurred spores. Sydowia 33: 111-144.

Hosen MI, Li TH, Ge ZW, *et al.* 2016. *Lepiota bengalensis*, a new species with first generic report for Bangladesh. Sydowia 68: 187-192.

Justo A, Angelini C, Bizzi A. 2015. Two new species and a new record of *Lepiota* (Basidiomycota, Agaricales) from the Dominican Republic. Mycological Progress 14: 56.

Kirk PM, Cannon PF, Minter DW, *et al.* 2008. Ainsworth and Bisby's Dictionary of the fungi, Wallingford, International Mycological Institute.

Kumar TA, Manimohan P. 2009. The genus *Lepiota* (Agaricales, Basidiomycota) in Kerala State, India. Mycotaxon 107: 105-138.

Liang JF, Yang ZL. 2011. A new species of *Lepiota* (Agaricaceae) from south western China. Mycotaxon 117: 359-363.

Mahmood S, Rahman AU, Sajjad A. 2019. Assessment of 2010 flood disaster causes and damages in district Muzaffargarh, Central Indus Basin, Pakistan. Environmental Earth Sciences 78: 1-11.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Gateway Computing Environments Workshop: 1 – 8.

Munsell A. 1975. *Munsell soil color charts*. Macbeth Division of Kollmorgen Instruments Corporation, New York.

Nawaz M, Wahla AJ, Kashif MS, *et al.* 2017. Effects of exogenous nitrogen levels on the yield of rice grain in Sheikhupura, Pakistan. Pakistan Journal of Agricultural Research 30: 85-92.

Nawaz R, Khalid AN, Hanif M, *et al.* 2013. *Lepiota vellingana* sp. nov. (Basidiomycota, Agaricales) a new species from Lahore, Pakistan. Mycological progress 12: 727-732.

Niazi AR, Asif M, Izhar A, *et al.* 2021. A new species of *Lepiota* (*Agaricaceae*) from Punjab, Pakistan. Phytotaxa 511: 163-174.

Nickson RT, McArthur JM, Shrestha B, *et al.* 2005. Arsenic and other drinking water quality issues, Muzaffargarh District, Pakistan. Applied geochemistry 20: 55-68.

Orton PD. 1960. New check list of British Agarics and Boleti: part III. Notes on genera and species in the list. Transactions of the British Mycological Society 43: 159-384.

Pegler DN, Rayner RW. 1969. A contribution to the agaric fora of Kenya. Kew Bulletin 23: 347-412

Qasim T, Khalid AN, Vellinga EC, *et al.* 2015. *Lepiota albogranulosa* sp. nov. (Agaricales, Agaricaceae) from Lahore, Pakistan. Mycological Progress 14: 24.

Qasim T, Khalid AN, Vellinga EC. 2016. A new species of *Lepiota*, *Lepiota lahorensis*, from Lahore, Pakistan. Turkish Journal of Botany 40: 419-426.

Razaq A, Khalid AN, Ilyas S. 2013. Molecular Identification of *Lepiota acutesquamosa* and *L. cristata* (Basidiomycota, Agaricales) based on ITS-rDNA barcoding from Himalayan Moist Temperate Forests of Pakistan. International Journal of Agriculture & Biology 15: 313-318.

Razaq A, Khalid AN, Vellinga EC. 2012. *Lepiota himalayensis* (Basidiomycota, Agaricales), a new species from Pakistan. Mycotaxon 121: 319-325.

Razaq A, Vellinga EC, Ilyas S, *et al.* 2014. *Lepiota brunneoincarnata* and L. subincarnata: distribution and phylogeny. Mycotaxon 126: 133-141.

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

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post–analysis of large phylogenies. Bioinformatics 30: 1312-1313.

Sysouphanthong P, Guo JY, Hyde K, *et al*. 2016. *Lepiota thailandica* (Agaricaceae) a new species from Thailand. Phytotaxa 245: 262-270.

Sysouphanthong P, Hyde KD, Chukeatirote E, *et al.* 2011. A review of genus *Lepiota* and its distribution in Asia. Current Research in Environmental & Applied Mycology 1: 161-176.

Sysouphanthong P, Thongklang N, Karunarathna SC, *et al.* 2020. *Lepiota condylospora*, a new species with nodulose spores in section *Lilaceae* from northern Thailand. Phytotaxa 455: 61-69.

Vellinga EC, Huijser HA. 1998. Studies in *Lepiota* I. Species with a hymeniform pileus covering. Belgian Journal of Botany 131: 191-210.

Vellinga EC, Sysouphanthong P, Hyde KD. 2011. The family Agaricaceae: phylogenies and two new white-spored genera. Mycologia, 103: 494-509.

Vellinga EC. 2001a. *Lepiota*. In: Noordeloos ME, Kuyper TW, Vellinga EC (eds.).Flora Agaricina Neerlandica 5. Swets & Zeitlinger Lisse. pp. 109-151.

Vellinga EC. 2001b. Studies in *Lepiota* IV. *Lepiota cristata* and *L. castaneidisca*. Mycotaxon 80: 297-306.

Vellinga EC. 2001c. Agaricaceae. In: Noordeloos ME, Kuyper TW, Vellinga EC (eds.). Flora Agaricina Neerlandica 5. Rotterdam, Balkema Publishers, pp. 220.

Vellinga EC. 2001d. Studies in *Lepiota* III. Some species from California, USA. Mycotaxon 80: 285-295.

Vellinga EC. 2003. Phylogeny of *Lepiota* (Agaricaceae) evidence from nrITS and nrLSU sequences. Mycological Progress 2: 305-322.

Vellinga EC. 2004. Ecology and distribution of lepiotaceous fungi (Agaricaceae) - a review. Nova Hedwigia 78: 273-299.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4239-4246.

Vizzini A, Liang JF, Jancovicová S. 2014. *Lepiota coloratipes*, a new species for *Lepiota rufipesss.* auct. europ. non ss. orig. Mycological Progress 13: 171-179.

White TJ, Bruns T, Lee S, *et al.* 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) PCR protocols: a guide to methods and applications. New York, Academic Press. pp. 315-322.

Yu WJ, Chang C, Qin LW, Zeng NK, Wang SX, Fan YG. 2020. *Pseudosperma citrinostipes* (*Inocybaceae*), a new species associated with Keteleeria from southwestern China. Phytotaxa 450: 8-16.

Zamir UB, Kazmi JH. 2014. Automated method for delineating watershed, drainage pattern and calculation of flow accumulation in Punjab province using digital elevation model. Pakistan Journal of Scientific & Industrial Research Series A: Physical Sciences 58: 90-98.