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Limacella subtropicana (*Amanitaceae, Agaricales*), a new species from Pakistan based on morphological and molecular phylogenetic evidences

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Abstract: Basidiomata of a hitherto undescribed *Limacella* species were collected from the grasslands of Punjab, Pakistan. We describe it as *Limacella subtropicana*, which is characterized by its viscid, white to creamy pileus, white to the creamy stipe, changing to brown when bruised, subglobose basidiospores measuring 4.0–6.3 × 3.7–5.5 µm, an ixotrichodermal pileipellis with subcylindric to clavate terminal elements and the common presence of clamp connections in all tissues. Maximum likelihood and Bayesian inference analyses of ITS sequences and LSU sequences of nrDNA confirmed its placement in the genus *Limacella*. A comprehensive description and a comparison of the new taxon with other *Limacella* species are provided.

Key words: Agaricoid, biodiversity, mycoflora, nonectomycorrhizal, taxonomy

1. Introduction

The genus *Limacella* s.l. is divided into three genera, namely *Limacella* s. str., *Limacellopsis* Zhu L. Yang, Q. Cai & Y.Y. Cui., and *Myxoderma* Kühner, which was changed to *Zhuliangomyces* Redhead by Redhead (2019) (Cui et al., 2018; Yang et al., 2018). Taxa included in *Limacella* s. str. are characterized by the agaricoid basidiomata, a glutinous or somewhat viscid, pallid to red-brown-colored pileus lacking remnants of a universal veil, free lamellae, a white to creamy spore print, and a viscid or dry stipe with a rudimentary annulus. Micromorphologically, the species within *Limacella* are recognized by a combination of the following characteristics: an ixotrichodermal pileipellis, fertile lamella edge, and globose to ellipsoid, smooth to asperulate, acyanophilous, inamyloid basidiospores (Singer, 1986 Neville and Poumarat, 2004; Ersel et al., 2005; Tulloss et al., 2016; Nascimento and Wartchow, 2018; Cui et al., 2018; Yang et al., 2018).

Worldwide, there are reports of the occurrence of 70 named members referring to approximately 48 different species of the genus *Limacella* s.l. Earle (1909: 447), most of those names are based on European and North American collections (Murrill, 1911; Smith, 1945; Pegler, 1983, 1986; Corner, 1994; Gminder, 1994; Kibby, 2004; Neville and Poumarat, 2004; Ferreira et al., 2013; Hosen and Li, 2017; Nascimento and Wartchow, 2018; Kumla et al., 2019; Yang et al., 2018; Usman and Khalid, 2020). *Limacella* species were also reported from Asia (Imai, 1938; Sathe and Daniel, 1981; Pegler, 1986; Corner, 1994; Yang and Chou, 2002; Sato et al., 2010; Yang, 2015; Cui et al., 2018; Usman and Khalid, 2020).

Knowledge of the genus *Limacella* s.l. in Pakistan is extremely limited, with only two species: *Zhuliangomyces illinitus* (Fr.) Redhead and *Z. pakistanicus* Usman and Khalid (Ahmad et al., 1997; Usman and Khalid, 2020) reported so far. The present contribution aims to propose a novel taxon collected from several areas of Punjab, Pakistan based on comprehensive morphological and molecular phylogenetic evidence.

2. Materials and methods

2.1. Locations and collections

Collections of basidiomata were made from three different areas of Punjab, Pakistan during field tours to various areas. The holotype was collected from district Sheikhupura, central Punjab (Coordinates: 31°42'47" N, 73°58'41" E, 236 m a.s.l) on 27 July 2017 during the monsoon season from loamy roadsides close to the Sheikhupura interchange, on motorway M2. Due to its fertile soil, the district is commonly named 'rice valley'. Climatic conditions of the district are categorized as subhumid with a maximum of 630 mm of annual rainfall (Sardar et al., 2013; Nawaz et

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al., 2017; Nabi et al., 2018; Shaheen et al., 2019; Sher et al., 2020; Alam et al., 2021). The common local vegetation of the area includes *Albizia lebbeck* (L.) Benth., *Capparis decidua* Pax., *Dalbergia sissoo* Roxb. *Ficus benghalensis* L. *Ficus religiosa* L., *Prosopis cineraria* (L.) Druce., *Salvadora oleoides* Decne., *Tamarix aphylla* (L.) Karst. Fl., *Vachellia modesta* Wall. and *V. nilotica* (L.) P.J.H. Hurter & Mabb. (Kazi, 1961).

Many collections under study were from Changa Manga Forest (Coordinates: 31°5'18" N, 73°5'52" E, 214 m a.s.l), which lies in a subtropical climate, on the southeast side of district Lahore, with annual temperature ranging from a minimum of 5.9 °C and a maximum of 39.6 °C and about 650 mm average annual rainfall. (Ashraf et al., 2010; Manzoor et al., 2010). The third locality was Muzaffargarh District (Coordinates: 30°4′10"N, 71°11'39"E, 65 m a.s.l) which is situated in south-western Punjab, an agriculturally important area (Zamir and Kazmi, 2014; Mahmood et al., 2019). The district receives 127–150 mm average annual rainfall (Nickson et al., 2005; Akram et al., 2014; Mahmood et al., 2019). Macro-morphological characters were noted from fresh basidiomata. Munsell's (1975) color chart was followed for color description. Samples were dried and processed in the laboratory. All collections were deposited in LAH Herbarium, University of the Punjab Lahore, Pakistan.

2.2. Microscopic study

Tissues of lamellae, pileus, and stipe were rehydrated in 5% KOH and stained with Congo red. Melzer's reagent was used to test the amyloid reaction of the basidiospores. The micro-characters observed include basidia, basidiospores, pileipellis, and stipitipellis (Vellinga, 2001b). All studies were made using an MX4300H compound microscope equipped with a camera Lucida 5x (Meiji Techno Co., Ltd. Japan). The symbolization "(n/m/p)" was used for measurements of basidiospore measurements, which means that n basidiospores, were measured from m basidiomata from p collections. The dimensions of basidiospores are presented in the form of (a) b–c (d) × (e) f–g (h), where b–c, f–g represent the basidiospores' length and width between the 5th and 95th percentile respectively, (a), (d) are smallest and largest recorded basidiospores whereas (e), (h) are narrowest and broadest basidiospores recorded. From three different collections, 60 basidiospores were measured from three basidiomata total. The following abbreviations are used: avl = average length, avw = average width, Q refers to the length/width ratio of basidiospores whereas Qav denotes the average of Q for basidiospores.

2.3. Molecular analysis

2.3.1. DNA extraction and amplification by PCR method

DNA was extracted from dried basidiomata according to a modified CTAB method (Zhao et al., 2011). The universal fungal primer pairs ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) for amplification of the ITS region and LROR and LR5 were used to amplify the LSU region of fungal DNA (Vilgalys and Hester, 1990). The PCR cycling to amplify the ITS region was according to Zhao et al. (2011) i.e. 5 min denaturation at 95 °C followed by 35 cycles of annealing at 94 °C (1 min), 1.5 min at 55 °C, 1.5 min at 72 °C and a final extension at 72 °C for 5 min. For PCR of the LSU region, initial denaturation at 94 °C for 2 min, 35 cycles at 94 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min, and final extension at 72 °C for 7 min. For visualization of PCR products, agarose gel electrophoresis was performed followed by visualization of amplified fragments in a gel documentation system (Avebury House, Cambridge, UK). Sequencing was performed by TsingKe, China with the same primers used for PCR amplification.

2.3.2. Phylogenetic analyses

Newly generated sequences were assembled by BioEdit software. ITS and LSU sequences of nrDNA, generated from the Pakistani collections were compared with sequences in GenBank using the BLAST tool, priority was given to those sequences which showed high bootstrap value in phylogenetic analyses (Altschul et al., 1990). The final datasets were created by adding newly generated sequences of *L. subtropicana* plus the highest scored hits were chosen from GenBank, also other members of the genus from previous studies (Cui et al., 2018; Usman and Khalid, 2020; Yang et al., 2018), the sequences with a negative E-value and with less query cover were excluded. *Amanita fulva* Fr. (KU139518, KU139519 for ITS; KU139448, KU139446 for LSU) was selected as outgroup (Figures 1 & 2). Alignment of the ITS and LSU datasets was constructed separately, using MUSCLE alignment software, then was manually adjusted in BioEdit where necessary (Hall, 1999). Both nucleotide alignments were deposited in TreeBASE (29482, 29483) as Nexus files. To compute the best fit model of nucleotide evolution, the nrITS dataset was subdivided into three partitions, ITS1, 5.8S, and ITS2. The best fit model of nucleotides substitution based on the lowest BIC (Bayesian information criterion) values for each partition and for nrLSU based dataset was chosen with jModelTest2 on XSEDE via CIPRES science gateway (Darriba et al., 2012). Maximum likelihood analysis was performed using RAxML-HPC2 v8.2.4 (Stamatakis, 2014) as implemented on the CIPRES portal with 1000 rapid bootstrap iterations for both datasets (Miller et al., 2010). Phylogenetic trees generated by Bayesian inference (BI) analyses were performed with a Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.8.2 (Drummond and Rambaut, 2007). Both analyses resulted in a similar topology. For the tree prior, a Yule-type speciation model (Gernhard, 2008) was used in all simulations, and the starting tree was randomly generated. Six independent runs were undertaken. The chain length was 20 million generations, with a sampling

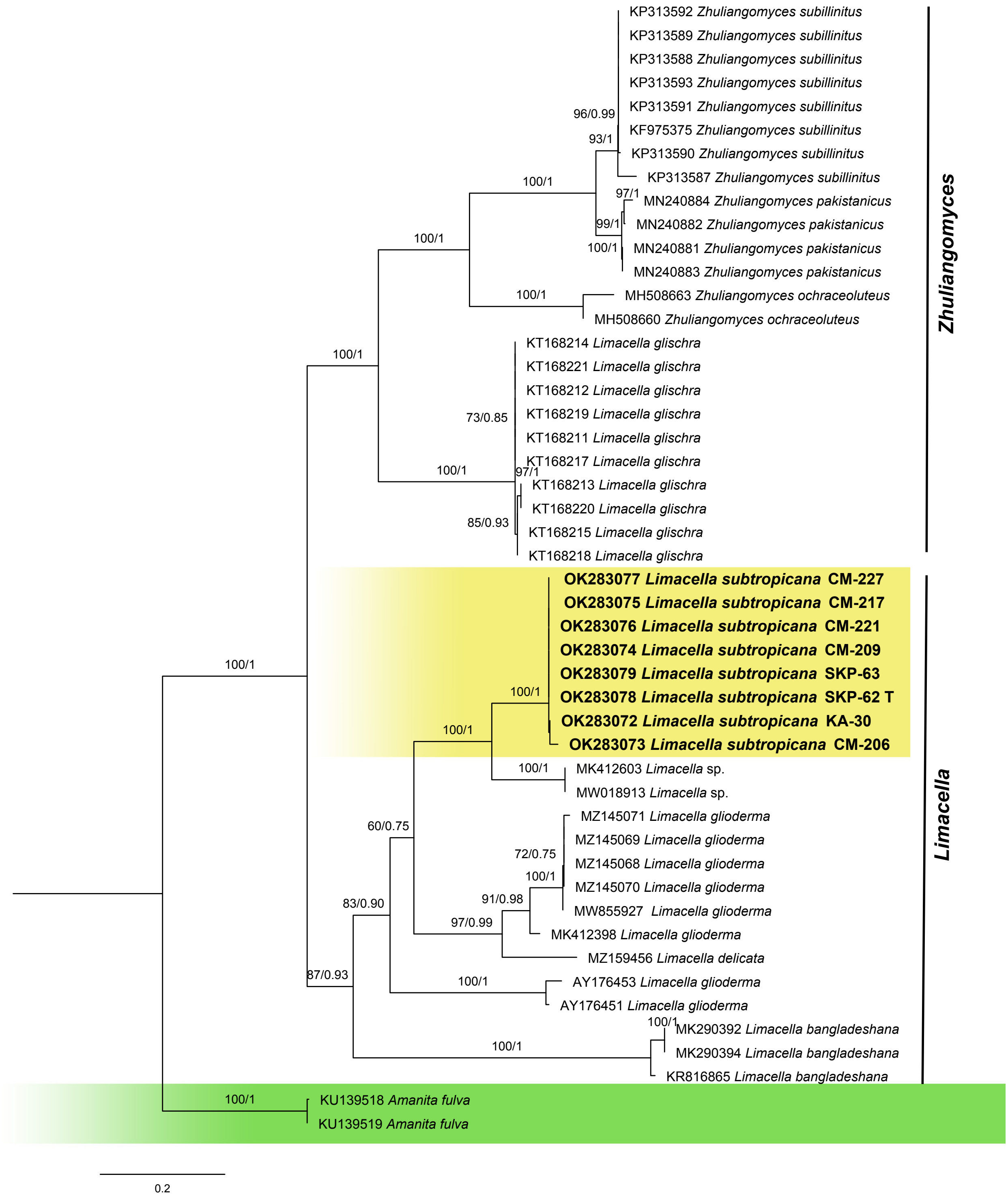

Figure 1. Molecular phylogenetic placement of *Limacella subtropicana* based on maximum likelihood (ML) method of ITS sequences. Newly generated sequences are in bold. *Limacella subtropicana* (LAH35706, T = Type specimen) is referring to the holotype. Bootstrap values > 70% and Bayesian posterior probabilities > 0.7 are shown above the branches.

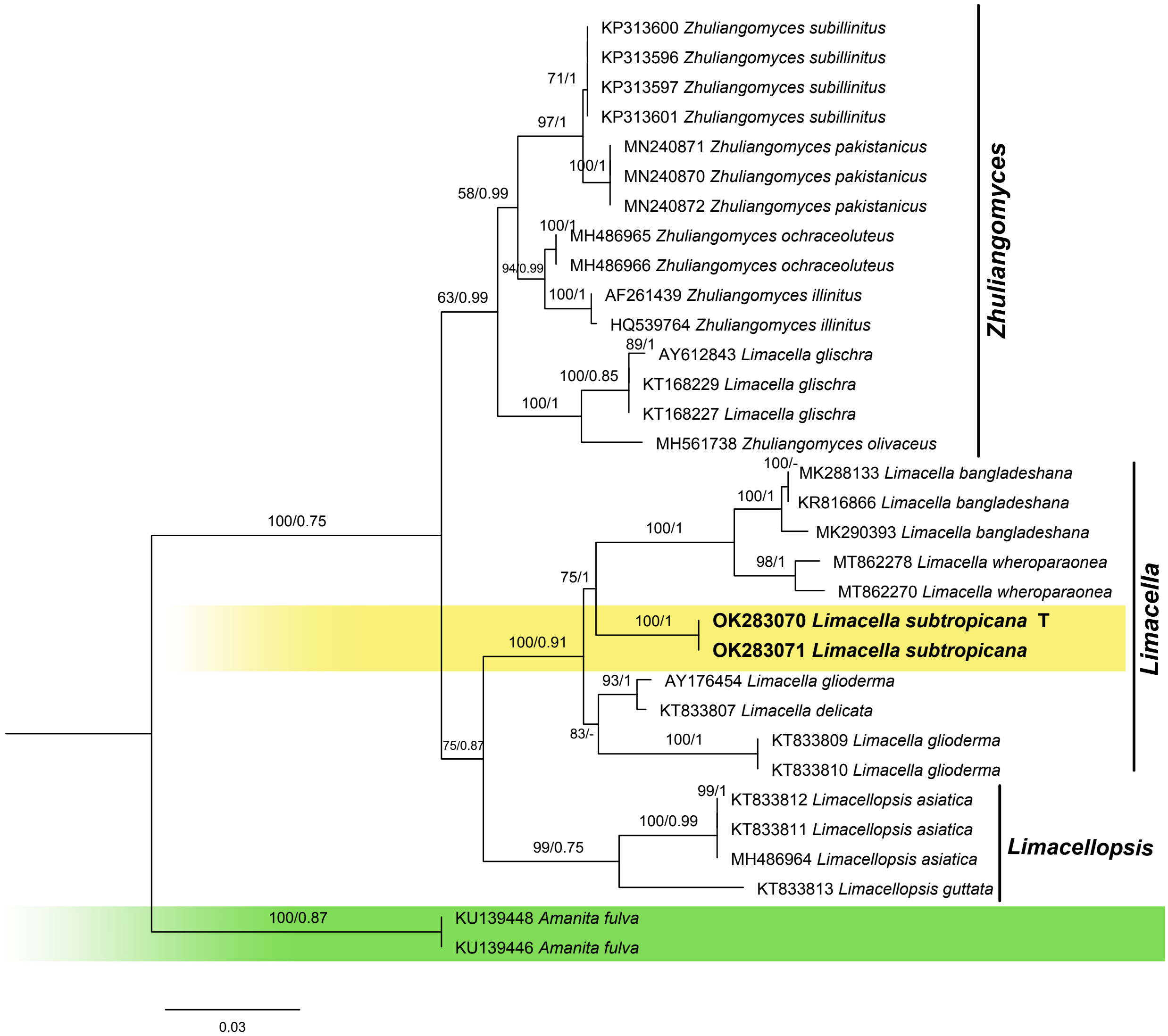

Figure 2. Molecular phylogenetic placement of *Limacella subtropicana* based on maximum likelihood (ML) analysis for LSU sequences. Newly generated sequences are in bold. *Limacella subtropicana* (LAH35706, T = Type specimen) is referring to the holotype. Bootstrap values > 50% and Bayesian posterior probability > 0.7 are shown above the branches.

frequency of 1000. Burn-in values were determined in Tracer 1.6 (Rambaut et al., 2014) with effective sample sizes (ESS) higher than 200. The maximum clade credibility (MCC) tree (20% burn-in value) was generated using TreeAnnotator 1.8.2 (Drummond and Rambaut, 2007). The output phylograms were displayed in FigTree 1.4.3 (Rambaut, 2014). Bootstrap values greater than 50% from RAxML are indicated. In the resulting trees, bootstrap values obtained from maximum likelihood analyses and values of Bayesian posterior probabilities greater than 0.7 were reported. Final phylograms were edited using Adobe Illustrator CS5.1 (San José, California). Newly obtained sequences of Pakistani collections were deposited in GenBank (Table 1) and a short description of the new species was deposited in MycoBank.

3. Results

3.1. Taxonomy

Limacella subtropicana A. Izhar, Niazi, M. Asif, Haqnawaz, H. Bashir, & Khalid, *sp. nov.*

Figures 3, 4

MycoBank No.: MB841277

Etymology: "*subtropicana*" (Lat.) is referring to the distribution of this species in subtropical areas.

Table 1. ITS dataset (Figure 1) and LSU dataset (Figure 2) used in the current study on *Limacella*. New species and its sequences produced in our study are in boldface.

Taxon	Voucher No.	GenBank Accession No.		Country
		ITS	LSU	
Limacella bangladeshana	HKAS:75316	KR816865	KR816866	Bangladesh
Limacella bangladeshana	CMU-JK0146	MK290394	MK290393	Thailand
Limacella bangladeshana	CMU-NK0356	MK290392	MK288133	Thailand
Limacella delicata	K(M):176976	MZ159456	-	UK
Limacella delicata	ZT: Myc55818	-	KT833807	Switzerland
Limacella glioderma	E.C. Vellinga 2456	AY176453	AY176454	USA
Limacella glioderma	E.C. Vellinga 2241	AY176451	-	Netherlands
Limacella glioderma	GLM:GLM-F51951	MK412398	-	Germany
Limacella glioderma	HBAU15753	MW855927	-	China
Limacella glioderma	HBAU15612	MZ145071	-	China
Limacella glioderma	HBAU15533	MZ145070	-	China
Limacella glioderma	HBAU15532	MZ145069	-	China
Limacella glioderma	HBAU15419	MZ145068	-	China
Limacella glioderma	MB 000750	-	KT833809	Germany
Limacella glioderma	MB 102389	-	KT833810	Germany
Limacella glischra	RET 540-4	KT168218	-	USA
Limacella glischra	Kuo-07020702	KT168216	KT168227	USA
Limacella glischra	RET 540-8	KT168217	-	USA
Limacella glischra	RET 540-3	KT168211	-	USA
Limacella glischra	RET 540-6	KT168219	-	USA
Limacella glischra	Kuo-08260501	KT168215	-	USA
Limacella glischra	RET 502-3	KT168220	KT168223	USA
Limacella glischra	RET 509-9	KT168214	-	USA
Limacella glischra	RET 540-2	KT168212	-	USA
Limacella glischra	RET 502-8	KT168221	-	USA
Limacella glischra	RET 540-7	KT168213	KT168229	USA
Limacella glischra	VTGB505	-	AY612843	USA
Limacella sp.	HAW:JKS61	MK412603	-	USA
Limacella sp.	iNAT:3196331	MW018913	-	USA
Limacella subtropicana	**LAH36973**	**OK283079**	**OK283071**	**Pakistan**
Limacella subtropicana	**LAH36972**	**OK283072**	-	**Pakistan**
Limacella subtropicana	**LAH2016206**	**OK283073**	-	**Pakistan**
Limacella subtropicana	**LAH2016208**	**OK283074**	-	**Pakistan**
Limacella subtropicana	**LAH2016216**	**OK283075**	-	**Pakistan**
Limacella subtropicana	**LAH2016221**	**OK283076**	-	**Pakistan**
Limacella subtropicana	**LAH2016227**	**OK283077**	-	**Pakistan**
Limacella subtropicana **(T)**	**LAH35706**	**OK283078**	**OK283070**	**Pakistan**
Limacella wheroparaonea	PDD:113481	-	MT862270	New Zealand
Limacella wheroparaonea	PDD:105537	-	MT862278	New Zealand
Limacellopsis asiatica	HKAS:76497	-	KT833811	China

Table 1. (Continued.)

Limacellopsis asiatica	HKAS:82561	-	KT833812	China
Limacellopsis asiatica	HKAS101436	-	MH486964	China
Limacellopsis guttata	MB 100157	-	KT833813	Germany
Zhuliangomyces illinitus	SFSU HDT 54917	-	HQ539764	USA
Zhuliangomyces illinitus	VT8.9.96	-	AF261439	USA
Zhuliangomyces ochraceoluteus	MEL2305332	MH508660	MH486965	Australia
Zhuliangomyces ochraceoluteus	MEL2341329	MH508661	MH486966	Australia
Zhuliangomyces olivaceus	HKAS101960	-	MH561738	China
Zhuliangomyces pakistanicus	LAH36341	MN240883	MN240871	Pakistan
Zhuliangomyces pakistanicus	LAH35337	MN240881	-	Pakistan
Zhuliangomyces pakistanicus	LAH36353	MN240884	MN240872	Pakistan
Zhuliangomyces pakistanicus	LAH35338	MN240882	MN240870	Pakistan
Zhuliangomyces subillinitus	RET 520-6	KP313587	-	USA
Zhuliangomyces subillinitus	RET 158-10	KP313590	-	USA
Zhuliangomyces subillinitus	NY 00027729	KF975375	-	Mexico
Zhuliangomyces subillinitus	RET 159-5	KP313593	KP313596	USA
Zhuliangomyces subillinitus	RET 159-2	KP313591	KP313601	USA
Zhuliangomyces subillinitus	RET 019-6	KP313588	KP313600	USA
Zhuliangomyces subillinitus	RET 159-3	KP313592	KP313597	USA
Zhuliangomyces subillinitus	RET 158-6	KP313589	-	USA
Outgroup				
Amanita fulva	ASIS26388	KU139518	KU139448	Korea
Amanita fulva	ASIS26398	KU139519	KU139446	Korea

Diagnosis: *Limacella subtropicana* is closely related to *L. whereoparaonea* G.S. Ridl., *L. pitereka* Grgur, and *L. glioderma*. However, it differs in: pileus viscid, white to creamy with a brown or brownish disc; stipe dry, white to creamy with dense brown fibrils, changing to brown on bruising; subglobose spores, 4.0–6.3 × 3.7–5.5 µm; pileipellis an ixotrichoderm with subcylindric terminal cells; clamp connections common.

Type: PAKISTAN. Punjab Province, Sheikhupura, on loamy soils, along roadsides of motorway M2, 31°42'40"N, 73°59'16"E, 236 m a.s.l., 27 July 2017, *Aiman Izhar*, *Skp-62* (holotype: LAH35706), GenBank: OK283078 for ITS, OK283070 for LSU.

Description: Basidiomata medium-sized. **Pileus** 40–60 mm in diameter, hemispherical when young, applanate at maturity, sometimes broadly umbonate; surface slimy and viscid, white to creamy (7.5YR512), subglabrous, covered with brown (5YR2/6) to brownish (10YR 3/4) cuticle, which may be ruptured into furfuraceous scales (Figures 3A, B). **Lamellae** free, white to creamy (2.5YR 9/2), with a pinkish tinge, regular, moderately thick, subdistant to close; edge entire to serrate, few slightly eroded; lamellulae present in 1–3 tiers (Figure 3C). **Stipe** 40–55 × 4.0–7.0 mm, centrally attached, white to creamy, subequal, cylindrical, slightly broader near the base, dry, densely covered with dark brown (5YR 2/6) to brown (10YR 3/4) fibrils on white background, becoming brownish when bruised (Figure 3D). **Annulus** present, superior, membranous to peronate, nongelatinous, often incomplete, or an appressed annular zone on the stipe. **Volva** absent. Odor mealy and taste not checked.

Basidiospores [60/3/3] (4.0–) 5.3–5.6 (–6.3) × (3.7–) 4.3–4.7 (–5.5) µm, avl × avw = 5.7 × 4.5 µm, Q = 1.1–1.15, Qav = 1.13, subglobose, a few broadly ellipsoid, apiculate, smooth, thin to moderately thick-walled, hyaline, inamyloid, nondextrinoid, guttulate (Figure 4A). **Basidia** 21–30 × 6.0–9.8 µm, thin to slightly thick-walled, clavate to subclavate, hyaline, frequently tetrasporic, rarely bisporic; basal septum in most cases with clamp-connection (Figure 4B). **Cheilo**-and **pleurocystidia** absent. **Pileipellis** an ixotrichoderm made up of pale to brownish filamentous hyphae, 3.0–5.0 µm in width, abundantly branched, smooth, frequently with clamp-connections, embedded in the gelatinous matrix; terminal cells 12–45 × 3.5–7.0 µm, subcylindrical to clavate, some narrowly conical or fusiform, mostly curved or hooked

Figure 3. Basidiomata of *Limacella subtropicana,* A and C = SKP-62 (Holotype); B and D = KA-30, Scale Bars = 10 mm. Photos by Aiman Izhar & Muhammad Haqnawaz.

(Figure 4C). **Stipitipellis** a cutis with hyphae 2.0–3.7 µm wide, hyaline with pale yellow to brown pigmentation, mostly clavate, few inflated, septate, branched; clamp connections frequent (Figure 4D).

Known distribution: In the plains of Punjab Province, Pakistan.

Additional materials examined: Pakistan. Punjab, Sheikhupura, on nutrient rich soils of motorway M2, (31°42'40"N, 73°59'16"E) 236 m a.s.l., 02 August 2018, *Aiman Izhar*, *Skp-63* (LAH36973); *ibid.*, Changa Manga (31°5'19.32"N, 73°57'44.76E, at 192 m a.s.l., solitary or in small groups on soil, 25 August 2016, *Abdul Rehman Niazi* & *Hira Bashir CM-206* (LAH2016206); 25 August 2016, *CM-209* (LAH2016208); 26 August 2016, *CM-217* (LAH2016216); 16 August 2017, *CM-221* (LAH2016221); 28 August 2018, *CM-227* (LAH2016227); *ibid.*, Muzaffargarh, solitary on nutrient-rich soil, 30°4'27"N, 71°11'4"E, 65 m a.s.l., 08 August 2019, *Muhammad Haqnawaz*, *KA-30* (LAH36972).

3.2. Molecular phylogenetic analyses

The newly generated ITS sequences of *Limacella subtropicana* were compared with those available in GenBank and published data that indicated it as distinct with 85% similarity to sequences of *Limacella* sp. (MW018913, MK412603). A total of 48 ITS sequences were assembled for phylogenetic reconstruction,

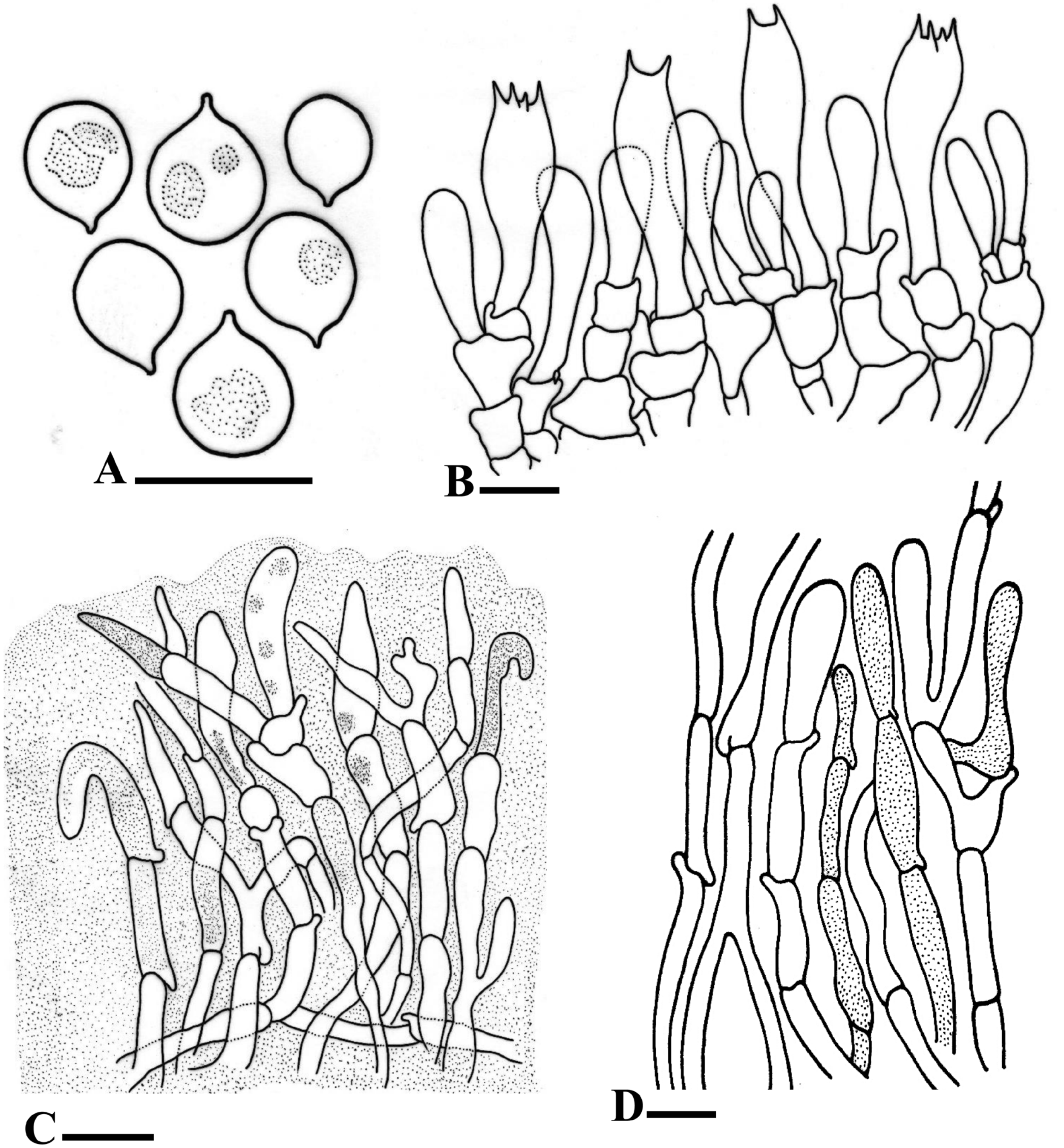

Figure 4. Micromorphological characters of *Limacella subtropicana*. A–D (SKP-62, Holotype): A = Basidiospores, B = Basidia, C = Ixotrichodermal pileipellis, D = Stipitipellis hyphae. Scale Bars = 10 µm. Drawings by Aiman Izhar.

representing 24 sequences from the genus *Limacella*, twenty-two from *Zhuliangomyces,* and two outgroup taxa. The best evolutionary models selected for the nrITS dataset were HKY+G+I (ITS1), TPM1uf+I+G (5.8S), and TPM3uf+I+G (ITS2). Two main clades were assigned according to previous phylogenetic work (Figure 1) (Cui et al., 2018; Yang et al., 2018; Usman and Khalid, 2020).

The LSU dataset (Figure 2) was constructed with sequences that were downloaded after BLAST, based on similarity, and from some recent publications (Cui et al., 2018; Yang et al., 2018; Usman and Khalid, 2020). There were 32 sequences in the combined tree, along with sequences of the new species. The same outgroups were used in the second analysis as in the first one.

After alignment with the online MUSCLE tool, the final alignment was 943 bp in length (including introduced gaps), of which 713 were constant, 218 variable, and 202 parsimony informative. For the second whole dataset, the TIM2+G model was selected as the best fit model for the phylogenetic analyses based on the lowest BIC values of 6959.053174. The clades were labeled according to Cui et al. (2018), Yang et al. (2018), and Usman and Khalid (2020). Sequences of *Catatrama* Franco-Molano (1991: 501) were not included as LSU sequences of its species were grouped with *Limacella* s.l. but with very weak bootstrap support. ML and Bayesian analyses resulted in identical topology, so, we have only presented phylogenetic trees resulting from ML analysis, combined with Bayesian posterior probabilities. Both phylogenetic trees showed that the proposed new species formed a monophyletic entity with strong bootstrap support.

4. Discussion

In this study, we introduce a new species, *L. subtropicana,* based on its macro- and micro-morphological features assisted by molecular phylogenetic studies. This is the third species of limacelloid fungi reported so far from Pakistan.

In the initial BLAST analysis based on ITS sequences of nrDNA, *L. subtropicana* showed 85% similarity to unpublished sequences named *Limacella* sp. (MW018913, MK412603) from USA. In the phylogenetic tree (Figure 1), these species are separated as independent species by 100% bootstrap support. The morpho-anatomical details of the voucher of *Limacella* sp. (MW018913, MK412603) are not available, and, therefore, a comparison with this species is not possible.

A commonly occurring species of *Limacella*, *L. glioderma,* previously reported from China, Europe, the USA, and East Asia is also close to our species in both of our phylograms. However, *L. glioderma* produces a red to yellowish-brown convex to plano-convex pileus with nonappendiculate to nonstriate margin, significantly longer stipe (up to 100 mm) covered with yellowish to red-brown squamules (Weiß et al., 1998; Neville and Poumarat, 2004; Kuo, 2017; Cui et al., 2018).

Our phylogenetic analyses of combined ITS and LSU sequences data (Figure 2) showed that *L. subtropicana* has a close relationship with *L. bangladeshana* described by Hosen and Li. (2017). However, *L. bangladeshana* differs in a caespitose habit, nonstriate pileus margin with orange to brown zone at the edge, no color changes in stipe when bruised, smaller basidiospores (3.5–4.0 × 3.5–4.0 µm) and longer terminal cells of pileipellis elements (25–70 µm long).

Other closely related species such as *Limacella whereoparaonea* and *L. pitereka* are distinguished from *L. subtropicana* as follows. *Limacella whereoparaonea,* a species originally reported from New Zealand, appears to be the closest species to *L. bangladeshana* in morpho-anatomical features. *Limacella whereoparaonea* differs from *L. subtropicana* by the presence of (rostrate, flexuous, or bifurcate) pleurocystidia and nonspecialized terminal cells of pileipellis (Ridley, 1993). *Limacella pitereka,* an Australian species, has an orange-tan to pinkish tint over the disc, proportionally longer basidiospores (4.6–8.0 µm), and longer basidia (up to 44 µm long) (Tulloss et al., 2021).

Several Asian species like *L. myxodictyon* (Berk. & Broome) Pegler, *L. magna* B. Kumaria & R.C. Upadhyay, and *L. anomologa* (Berk. & Broome) Pegler, have morpho-anatomical similarities to our species but have many clear differences. A Sri Lankan species *L. myxodictyon*, contrasts from *L. subtropicana* by its dark reddish-brown pileus, globose small sized basidiospores of 3.5–5 × 3–4 µm (Pegler, 1986). An Indian species *L. magna* produces significantly larger pileus (200 mm broad), capucine buff surface with brown fibrillose squamules, adnexed lamellae, a much larger stipe (220 × 25–27 mm), mostly globose much bigger (7.0–14.0 × 5.0–7.0 µm), amyloid basidiospores and a pileus covering composed of many lageniform elements (Kumari et al., 2013). Another Sri Lankan species *Limacella anomologa* can be distinguished by the quite different blackish olivaceous color at the pileus center becoming pale green towards the margin, a longer stipe (up to 80 mm long), and a glutinous zone at the half-length of the stipe (Pegler, 1986).

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