Lepista irina: A New Record for a Wild Edible Mushroom in Pakistan, Authenticity Validated by Molecular and Morphological Evidence

Ishtiaq Ahmad[*,], Malka Saba[***], Andrew N. Miller[*] and Abdul Nasir Khalid[****]**

[*] *Illinois Natural History Survey, University of Illinois Urbana-Champaign, Champaign, IL, USA*
[**] *Department of Botany, Islamia College, Peshawar, Pakistan*
[***] *Department of Plant Sciences, Quid E Azam University, Islamabad, Pakistan*
[****] *Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus 54590, Lahore, Pakistan.*

Abstract- *Lepista irina* (Fr.) H. E. Bigelow is a common edible mushroom belonging to the Tricholomataceae family (Agaricales; Basidiomycota). This species has been recorded for the first time in Pakistan as a result of an extensive biodiversity assessment of the mushrooms of the Shangla region of Khyber Pakhtunkhwa. We present an in-depth phylogenetic analysis of the nrDNA ITS sequences along with micro- and macro-morphological descriptions for the authentic taxonomic placement of this species.

Index Terms- Edible, Mushrooms, Pakistan, Phylogenetics

I. INTRODUCTION

The fact that people have long used mushrooms as food, medicine, and a source of revenue has been widely acknowledged in the mycological literature (Buyck and Nzigidahera, 1995; Tibuhwa, 2012; Pérez-Moreno *et al*., 2021). There are more than 2000 species of edible mushrooms found in the world, however, only 257 are regularly farmed and consumed as food for humans (Li *et al*., 2021). Edible mushrooms are the top-ranked organic products due to their unique nutritional composition. They contain the highest levels of carbohydrate and protein (1.5–6.7% and 1.5–3%, respectively), low levels of fat (0.3–0.4%), and a high concentration of vitamins (B2, B3, and D) and minerals such as potassium, phosphorus and magnesium, etc. (Barros *et al*., 2007).

Among the gilled fungi, the Agaricaceae, Bolbitiaceae, Lepiotaceae, and Tricholomataceae families contain the majority of the edible species of mushrooms. One important genus among them is *Lepista* (Fr.) W. G. Smith (Tricholomataceae), which includes edible species like *L. irina*, *L. nuda*, *L. personata*, and *L. sordida*. (Buczacki, 1992; Stott *et al*., 2005). *Lepista* species have a considerable economic potential, which is demonstrated by the consumption of *L. nuda*, the second most frequently consumed edible gilled fungus in Western Europe (Lannoy, 1982; Stott *et al*., 2005; Marshall and Nair, 2009).

In the field, *Lepista* species may be identified as tricholomatoid or clitocyboid agarics with gills that are easily separable from the cap and stem. The lepistas are further distinguished by a variety of colors, scents, and growth patterns. The characteristics of basidiospores and basidia also play a significant role in species differentiation of Lepista species (Yoo, 2022). Previous reports from Pakistan list the following six species of *Lepista*: *L. caffrorum* (Kalchbr and McOwen) Sing., *L. inversa* (Scop.) Pat = *Paralepista flaccida* (Sowerby) Vizzini, *L. luscina* (Fr.) Sing., *L. nuda* (Bull.) Cooke., *L. obscura* (Sch.) Herink = *L. panaeolus* (Fr.) P. Karst., and *L. sordida* (Schum.: Fr.) Sing. (Ahmad, 1980; Murakami, 1993; Iqbal and Khalid, 1996; Sultana *et al*., 2011). This research extends the total number of *Lepista* species recognized in Pakistan to seven.

II. MATERIALS AND METHODS

Basidiomata were collected from Yakh Tangay, which is located at an elevation of 6470 feet, and Shangla Top, which is at an elevation of 7055 feet, within the district of Shangla. Basidiomata were photographed with a DSLR (Nikon D3500) camera and dried using a heating fan in the field. Microscopic examinations were made of sections of specimens mounted in 5% KOH using a Meiji MX4300H microscope (Meiji Techno Co., Ltd., Japan). Phloxine dye and Melzer's reagent were used to enhance the contrast of the structures and record the color reactions. The measurement of micro-structures like basidiospores, basidia, cystidia and cuticular hyphae of the stipe and pileus are based on a minimum of 20 measurements taken with an ocular micrometer under 100x power. Q = mean spore length divided by mean spore width. Illustrations were drawn manually using a camera lucida. Color values are derived from the Munsell color chart (Jabeen *et al*., 2019).

Genomic DNA was extracted from a small portion of the lamellae using a modified CTAB method (Bruns, 1995). The REDTaq ReadyMix PCR Reaction Mix was used for the amplification of the complete nrITS region (White *et al*., 1990; Gardes and Bruns, 1993) using the ITS1F/ITS4 primers and #µL of DNA. The following PCR cycling temperatures

were used: initial denaturation at 94 °C for 1 min followed by 35 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 8 min. Successful PCR products were purified and sequenced in both directions with the same primers as above at a commercial facility (Macrogen Korea).

The closely matching sequences of *L. irina* were downloaded from NCBI and aligned with our sequences using the online version of MUSCLE (Tamura *et al*., 2011). Sequences were manually edited and reassembled using BioEdit. All DNA sequences were trimmed to include the motifs 5'-(...GAT) CATTA- and -GACCT (CAAA...)-3' in accordance with Dentinger et al. (2011) for the entire ITS region prior to analysis. The phylogenetic tree was created using the latest version of MEGA software with default preferences. A Maximum Likelihood (ML) algorithm was employed using the Jukes Cantor (1969) model and the nearest-neighbor-interchange (NNI) ML heuristic search (Tamura *et al*., 2021). The tree includes relevant bootstrap values >50% after 1,000 repetitions that were used to evaluate support for clades. Table 1 lists the origin and GenBank accession numbers for all taxa used in the phylogenetic analysis. DNA divergence and percent identities (PID) were calculated by DNAStar Inc (Sarwar *et al*. 2018).

III. RESULTS

Lepista irina (Fr.) H.E. Bigelow. *Can. J. Bot*. 37: 775 (1959). (Figs 1–2)

≡ *Gyrophila irina* (Fr.) Quél., Enchir. fung. (Paris): 17 (1886)
≡ *Lepista irina* var. *montana* Bon, Bull. trimest. Féd. Mycol. Dauphiné-Savoie 25 (no. 97): 25 (1985)
≡ *Clitocybe irina* var. *luteospora* H.E. Bigelow & A.H. Sm., Brittonia 21: 174 (1969)

PILEUS 5–8 cm diam, hemispherical to broadly convex, expanding to plane, somewhat sunken central disc, whitish when younger, mild orange-colored to deep brownish orange when old (2.5YR5/8-5YR5/8-5YR6/8), surface smooth, glabrous, velvety; curvature deflexed to inrolled; margins rolled to cottony becoming degraded with maturity; context thick, soft, whitish or pinkish or watery avellaneous. LAMELLAE adnate, almost crowded, thin, light to dark orange (10R4/12-5YR5/8); ragged, eroded edges. STIPE 6.5–8.5 × 1–2 cm; soft, hollow, cylindrical; whitish to light brownish to mild brownish orange (5YR7/8-5YR5/8); surface reticulated to striated, pallid at first, more or less fibrillose to slightly scabrous, in age becoming sordid avellaneous.

BASIDIOSPORES (3.4–) 4–5 × 6–8 (–9.6) µm [Q = 1.625], ellipsoid to oblong, with an apiculus, mostly verruculose but some smooth, thin-walled, hyaline in KOH, non-amyloid. BASIDIA 7–10 × 23–31 µm, clavate, thin-walled, hyaline in KOH, 1–4 spored; sterigmata 2.5-3.5 µm. Tramal hyphae 5.5–9.5 µm diam, cylindrical to inflated, thin-walled, regular. Cystidia not observed. Pileipellis (3.5–) 5.5–9 µm, a cutis, cylindrical, thin-walled, hyaline. Stiptipellis 3–8 µm, cylindrical, hyaline to pale yellow. Clamp connections present.

Molecular Characterization and Sequence Analysis:
Sequences of ca. 700 base pairs were generated from five distinct collections of *L. irina*. The ITS sequences from our collections (ISP#005, ISP#010, ISP#011, ISP#012, and MS#006) exhibited 99% similarity with *L. irina* (GenBank accession HM237136), reported from New Zealand, according to an initial blast analysis of nucleotide sequences. Sequences of the closely related taxa were downloaded from Genbank and these, along with five *L. irina* sequences from Pakistan, were included in the final dataset of 25 sequences (Table 1). Based on the findings from Vizzini *et al*., (2012), *Xeromphalina campanella* (GU320006) was chosen as an outgroup. After manual correction of the alignment, the final dataset consisted of a total of 663 positions for the phylogenetic analysis. There were 469 conserved characters, 161 variable characters, 97 parsimony informative characters, and 59 singletons. The maximum likelihood analysis generated a single most-likely tree with a log likelihood value of -2271.0453 (Fig. 3). The same tree topology was produced with a maximum parsimony analysis.

Habit and Habitat: Solitary to sub-caespitose to densely gregarious, clustered in 2-4 basidiomata or dispersed, under *Pinus wallichiana* A. B. Jacks.

MATERIAL EXAMINED: Pakistan, KP, District Shangla, Shangla-Top & Yakh-Tangay, 02 Sep 2013, ISP # 0005(LAH & ICFP); ISP # 0010 (LAH & ICFP); ISP # 0011 (LAH & ICFP); ISP # 0012 (LAH & ICFP); MSM # 006 (LAH).

IV. DISCUSSION

The research being conducted focuses on confirming the identity of a wild edible mushroom found in Pakistan. *Lepista* is a small genus, which is mostly found in temperate regions, but may also be found in the tropics. It is widely recognized to contain many edible mushrooms, such as the wood blewit, *L. nuda* (Bull. ex. Fr.) Cooke and the blewit, *L. saeva* (Fr.) P. D. Orton in north temperate latitudes, and *L. caffrorum* (Kalchbr. & MacOwan) Singer in South Africa (Pegler and Young, 1974). *Lepista irina* is a popular edible species with a name sometimes referred to as flowery blewit since it has a characteristic floral odor.

Bigelow (1959) first provided a description of *L. irina* from Canada. It may be identified by a broadly convex pileus with a central disc that is somewhat depressed, deflexed edges, crowded gills that are adnate or emarginate, a central to eccentric stipe, basidiospores that range from being smooth to being minutely spiny or verrucae-like, and the existence of clamp connections in all tissues.

The current collections resemble the description given by Bigelow (1959), except that the basidia sometimes possess a single sterigmata. When examined under a light microscope, basidiospores were almost completely smooth, while some had minor verrucae when visualized under oil immersion. According to Pegler and Young (1974), all *Lepista* spores are adorned with solitary verrucae, however the decoration is typically difficult to identify using light microscopy due to their tiny size. The identification of *L. irina* from Pakistan is supported by both morphological and molecular evidence.

Lepista irina is similar to *L. nuda*, which was previously identified from Pakistan, in that it occurs in similar habitats.

The basidiocarp of *L. irina* lacks blue, lilaceous hues and has a unique odor. Additionally, it appears that *L. nuda* has more verrucae on its surface than *L. irina*. Two significant clades are recovered in the phylogenetic tree (Fig. 3). Clade A is featured by a number of other Lepista species from around the world. Whereas, clade B, characterized by a strong bootstrap support (100) shows the grouping of Pakistani taxa with other *L. irina* species retrieved from the GenBank (Table-1). It includes Lepista species originated from China, New Zealand, and the United States. The percentage similarity of *L. irina* assessed by DNAStar indicated >97% identity with a sequence received from GenBank (HM237136), which supports the findings shown by blast analysis and the phylogenetic tree.

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AUTHORS

First Author – Ishtiaq Ahmad, Ph.D. and postdoc, Illinois Natural History Survey, University of Illinois Urbana- Champaign, Champaign, IL, USA and Department of Botany, Islamia College, Peshawar, Pakistan.

Second Author – Malka Saba, Ph.D., *Department of* Plant Sciences, Quid E Azam University, Islamabad, Pakistan,

Third Author – Prof. Dr. Andrew N. Miller, Ph.D. and postdoc, Illinois Natural History Survey, University of Illinois Urbana- Champaign, Champaign, IL, USA,

Fourth Author – Prof. Dr. Abdul Nasir Khalid, Ph.D., Fungal Biology and Systematics Research Laboratory, Institute of Botany, University of the Punjab, Quaid-e-Azam Campus 54590, Lahore, Pakistan,

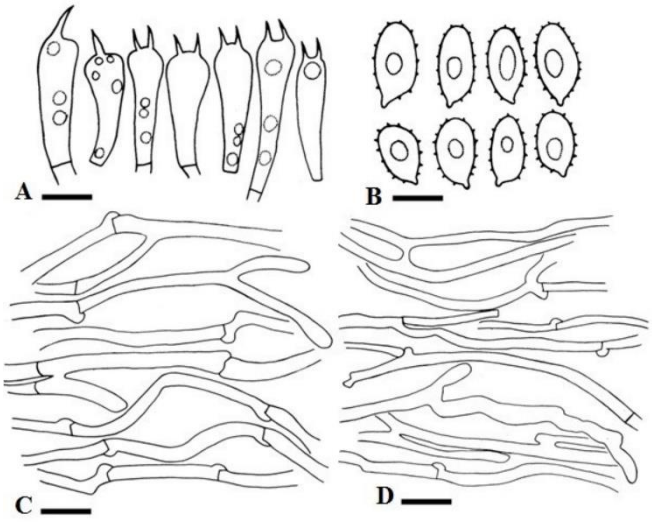

Fig. 1 A–D – Hand-drawings showing the internal structures of *Lepista irina*. A – Basidia, B – Basidiospores C – Pileipellis and D – Stipe elements. Scale bars: A = 7µm; B = 4.5µm: C-D = 21.5µm

Table 1. Origin, GenBank Accession No. and Voucher Number of the species used in phylogenetic analysis.

Taxa	Origin	Voucher Number	GenBank Accession No.
Lepista sordida	South Korea	IFO 31013	AF241523
Tricholoma mongolicum	China	HMJAU:24940	KC413943
Tricholoma mongolicum	China	HMJAU:24939	KC413942
Lepista nuda	China	GSM-11	JQ519375
Lepista nuda	USA	TRTC155545	JN021061
Collybia cirrhata	Canada	MD_HP06_95	JN021007
Collybia cirrhata	Canada	MD_HP06_95a	JN021006
Clitocybe paxillus	Netherlands	GG173_88	GU234065
Clitocybe paxillus	Netherlands	O50514	GU234110
Clitocybe dryadicola	Netherlands	GG51_88	GU234064
Clitocybe dryadicola	Netherlands	O50495	GU234101
Clitocybe nebularis	Slovenia	Vrh2004	DQ149728
Clitocybe nebularis	Slovenia	Kras2004	DQ149727
Lyophyllum fumosum	China	lfxq	HM119490
Lyophyllum fumosum	China	Lfwy	HM119489
Lepista irina	China	dd08025	FJ810142
Lepista irina	New Zealand	OTA61646	HM237136
Lepista irina	USA	PBM 2291	DQ221109
Lepista irina	Pakistan	*p-5*	KJ439560
Lepista irina	Pakistan	*P-10*	KJ396083
Lepista irina	Pakistan	*P-11*	KJ396084
Lepista irina	Pakistan	*P-12*	KJ396085
Lepista irina	Pakistan	*M-92*	KJ194172
Xeromphalina campanella	Spain	AH3922	GU320006

Fig. 2 E–G – Basidiomes of *Lepista irina*. Scale bars: E–G = 1 cm.

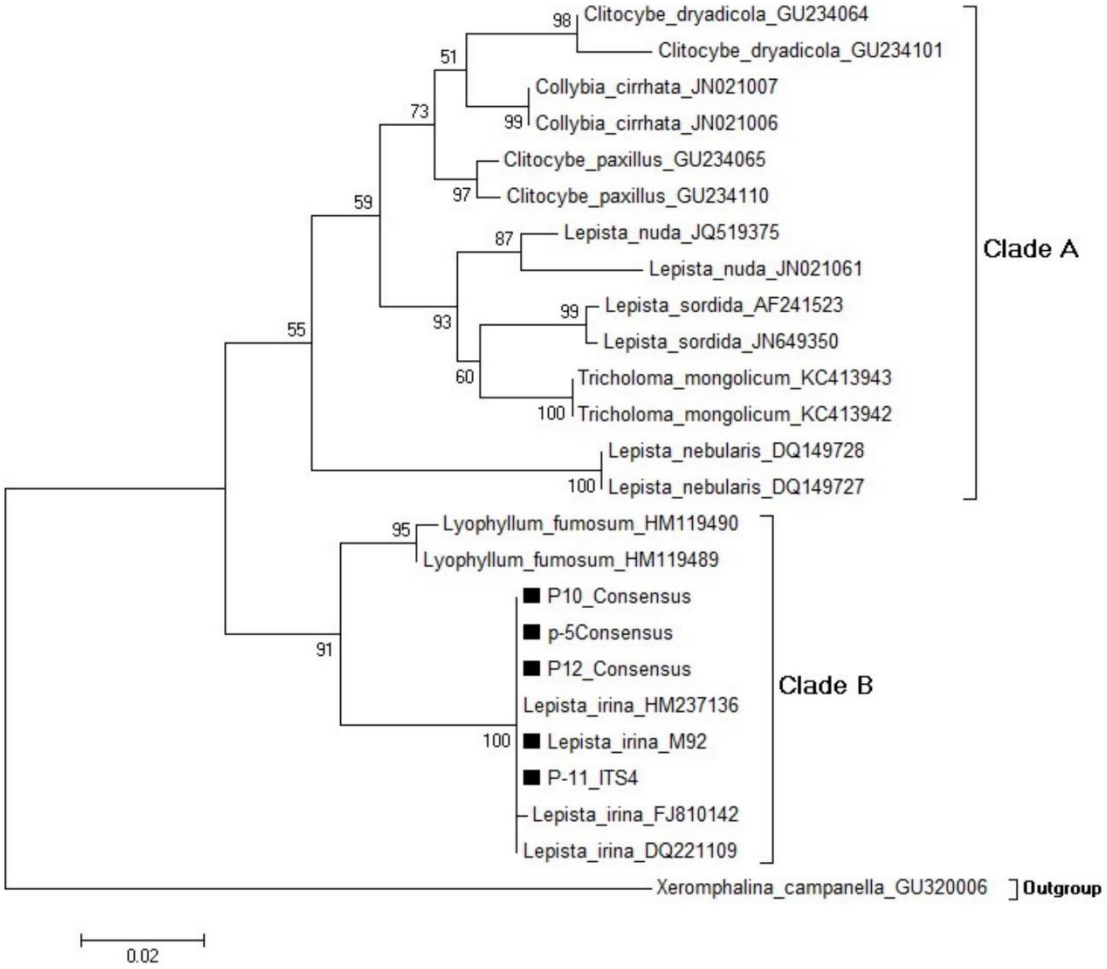

Fig. 3 – Phylogenetic relationships of *Lepista irina* (■) with other related members of Tricholomataceae based on Maximum Likelihood method inferred from nrITS sequences.