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Molecular phylogeny and morphological characterization of *Leucocoprinus birnbaumii* from Punjab, Pakistan

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Abstract

Background: *Leucocoprinus* in *Agaricaceae* is a genus distributed worldwide and represented by nearly 50 species. From Pakistan, only four species in this genus have been known so far.

Methods: The specimen was identified by morphological and anatomical characters combining with sequence analysis of nrDNA-ITS region.

Results: *Leucocoprinus birnbaumii*, a bright yellow mushroom found on wood log from Sialkot district, Punjab, Pakistan. This species is identified by its medium sized bell-shaped pileus bearing the same colored squamule with sulcate striate margins, presence of metachromatic basidiospores and pseudoparaphyses around the basidia. Molecular data also supported its taxonomy as *L. birnbaumii*. The species is being described for the first time based on morphological and anatomical characters in combination with molecular phylogeny using ITS region of nrDNA.

Conclusion: *Leucocoprinus birnbaumii* is widely distributed in Asia, Africa, Europe, North America and South America. It grows in tropical and sub-tropical areas. Its occurrence in Sialkot, Punjab, Pakistan with humid subtropical climate represents its wide ecological amplitude and geographic distribution.

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Humid subtropical climate; ITS; nrDNA-ITS; Taxonomy

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This article has post-publication language corrections.



Introduction

Leucocoprinus Pat. is a genus of lepiotaceous fungi in *Agaricaceae* distributed worldwide in the tropical and sub-tropical areas [1]. This genus is represented by nearly 50 species and typified by *L. cepistipes* (Sowerby) Pat [2-4]. It occurs naturally in Asia, Central and Southern Africa, Europe, North America and South America and mostly common in hot, humid weather [4-9]. From Pakistan, only four species in this genus have been known so far in the form of a list with material examined but their detailed descriptions are not given [10]. The members of the genus are saprobic and quickly grow in potting soils, greenhouses and man-made other organic rich matters [6, 9, 11, 12].

Leucocoprinus is characterized by pale, white, metachromatic basidiospores and the sulcate, often plicate pileus margins. These characters are intermediate between *Leucoagaricus* Locq. ex Singer and *Macrolepiota* Singer [13]. This difference was clarified by the discovery of presence of pseudoparaphyses between its basidia that are absent in *Leucoagaricus* species. Clamp connections are absent in all the tissues of *Leucocoprinus* [9, 11, 14].

The current study emphasizes on characterization of specimens found morphologically similar to *Leucocoprinus* growing on wooden logs in Sialkot district, Punjab, Pakistan. The detailed morphological and anatomical description and molecular phylogenetic analysis based on ITS region of nrDNA was carried out to identify the specimens up to species level.

Methods

The specimens were collected from Khajoriwala, Sialkot district, Gujranwala division, Punjab, Pakistan during the monsoon season of July 2019 and their macroscopic characters were noted. Colors were noted and codes were given according to the Munsell, 1975, [15]. Photographs were taken and then basidiomata were subjected to air dry for preservation. Microscopic structures were studied under the microscope (Xsz 107BN) with a 100× objective lens. All microscopic features including size, shape, color, and arrangements of hyphae in different tissues were noted. Measurements were recorded using Motic Images Plus 2.0. For the measurements of basidiospores, the notation [s/b/c] was used, where s represents the number of basidiospores, measured from b basidiomata and c collections. Extreme values of basidiospores dimensions (l × w) were given in parenthesis. Q values were calculated as l/w ratio. Q values for spores were interpreted following Bas (1969) [16]. Drawings were drawn from the laptop screen. The examined

specimens were deposited in the herbarium of University of Education, Lahore, Pakistan (UEH).

For genomic DNA, the Extract-N-Amp™ kit was used following the manufacturer's instructions. The ITS region was amplified and sequenced from the Sanger sequence using ITS1F (forward primer) and ITS4 (reverse primer) primers. Sequences obtained were analyzed in BioEdit sequence alignment editor version 7.2.5 [17]. The consensus sequence was BLAST searched at NCBI database (Nucleotide BLAST). Closely related sequences were selected from GenBank to reconstruct phylogeny. Published sequences of the closest relatives of the species were also included in the dataset. All the sequences were aligned online using MUSCLE [18] alignment tool. The final aligned data set was analyzed through MEGA 6.0 software for reassessing the phylogeny (Figure 1). Kimura 2-parameter [19] was selected as an evolution model for maximum likelihood analysis using the best-fit substitution model approach in MEGA [20].

Results

Molecular phylogeny

The total fragment size of the target region of nrDNA of collected specimen yielded 784 base pairs. The BLAST results showed 98.56–99.46% similarity with *Leucocoprinus birnbaumii* sequences (LN827701, U85323 and ON627825) from Egypt, UK and USA. These sequences along with other sequences from GenBank were retrieved for the phylogenetic analysis. *Leucoagaricus rubrotinctus* (Peck) Singer (KP300876) and *L. lahorensiformis* Hussain *et al.*, (KU647729) were used to root the tree following Justo *et al.* (2021) phylogeny [4].

The final dataset of ITS region contains 58 nucleotide sequences including outgroups representing 731 positions. Among these positions, 438 were conserved, 285 were variable, 238 were parsimony informative and 46 were found as singletons. In a phylogenetic tree, sequences are arranged to represent the evolutionary relationships among organisms or genes, where branch lengths often indicate genetic distance or time since divergence.

Taxonomy

Leucocoprinus birnbaumii (Corda) Singer, *Sydowia* 15(1-6): 67 (1962)

Description

Pileus up to 4.7 cm broad, campanulate, broadly umbonate, bright yellow (5Y8/10); fibrillose, floccose to loosely scattered concolorous squamules; margins incurved, sometimes straight, sulcate-striate, pale yellow (2.5Y8/8); context yellow (7.5Y 9/6). Lamellae free, sub-distant, ventricose, pale yellow (2.5Y8/8);

edges fimbriate. Lamellulae absent. Stipe 6.5–9 × 0.4–0.9 cm; central, equal, base bulbous upto 1.4 cm, pale yellow (7.5Y 9/3); surface floccose squamulose; light yellow (5Y8/10); context yellow (7.5Y9/6). Annulus central, moveable, uplifted, thin, band like; yellow (2.5Y8/12), leaving remnants on pileus margins. Volva absent.

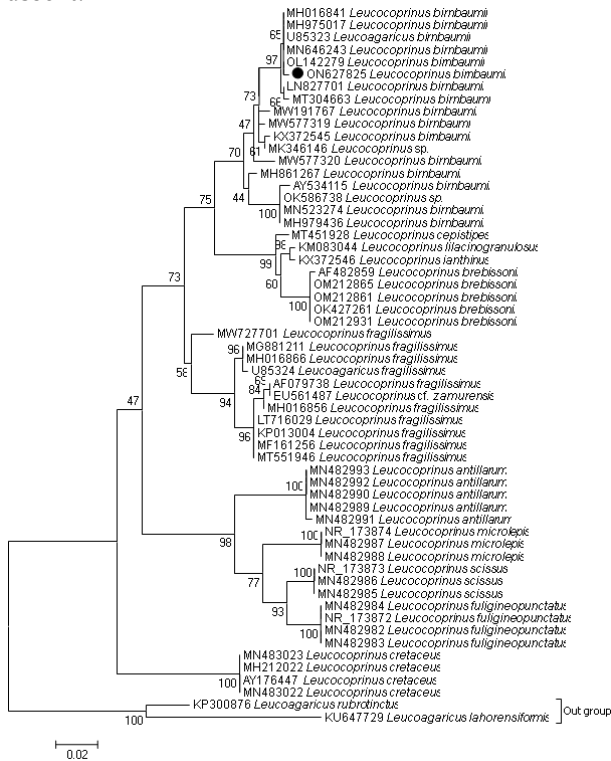


Figure 1: Phylogenetic analysis of ITS sequences

representing *Leucocoprinus birnbaumii* and related species in the genus along with two outgroup taxa. The sequence generated during this study is marked with the bullet.



Figure 2: Basidiomata of *Leucocoprinus birnbaumii* UEH-F0001 showing its morphological features. Scale bars = 1 cm.

Basidiospores [60/3/1] (10)10.1–11.7(12) × (6.9)7.2–8.3(8.4) μm, Q (1.4)1.41–1.43(1.44), avQ = 1.42, ellipsoid, amygdaliform, thick walled, apiculate with a prominent germ pore, smooth, metachromatic. Basidia (18.3)18.5–23.7(24.6) × (9.1)9.3–14(14.5) μm, cylindrical to clavate, 4-spored, thin walled, hyaline. Pseudoparaphyses (14.7)16–17(17.5) × (12.2)12.5–14.5(15) μm, broadly clavate, thin walled, hyaline. Cheilocystidia (45)48–50.6(54) × (9.4)11.0–11.7(14) μm, clavate to narrowly utriform or fusiform, flexuous, thin walled. Pleurocystidia absent. Stipitipellis hyphae (5)5.1–7(9) μm, cylindrical, septate, thin walled. Pileipellis hyphae (5.7)8–9.3(9.6) μm, cylindrical, thin walled. Clamp connections absent.

Material Examined: Pakistan, Punjab, Gujranwala division, Sialkot district, Khajoriwala, 256 m a.s.l. from wooden roof, 25 July 2019, Maham Mureed MM34-SJ220 (UEH-F0001; GenBank accession: ON627825).

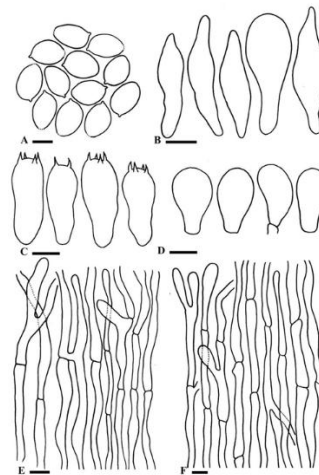


Figure 3: *Leucocoprinus birnbaumii* UEH-F0001. A. Basidiospores; B. Cheilocystidia; C. Basidia; D. Pseudoparaphyses; E. Stipitipellis; F. Pileipellis. Scale bars = 10 μm.

Discussion

Leucocoprinus birnbaumii is the best-known member of *Leucocoprinus*, previously placed in different genera; *Agaricus* L., *Bolbitius* Fr. and *Leptota* (Pers.) Gray and was known as *Agaricus aureus* F.M. Bailey and *A. birnbaumii* Corda. which are now reduced to synonyms (IndexFungorum) [8, 21]. It is commonly known as flowerpot parasol and the plantpot dapperling as it is usually found growing in flowerpots and greenhouses [22].

Leucocoprinus birnbaumii is identified by bright yellow, medium sized basidiomata having bell shaped pileus bearing same colored squamules with sulcate striate margins, presence of metachromatic basidiospores and pseudoparaphyses around the

basidia [5-9, 13]. It is an inedible, poisonous mushroom widely distributed in Asia, Africa, Europe and North and South America and grow on decayed plant matter, humus rich or composted soil in flowerpots and flowerboxes in green houses or in buildings in tropical and sub-tropical areas [1, 6, 8, 9, 11]. A series of alkaloids and fatty acids have been studied and identified in *L. birnbaumii* which are effective against bacterial activity [9, 22, 23].

he present collection was found solitary on a wooden log

from the humid subtropical climate (Köppen climate classification) of Gujranwala division, Sialkot district, Punjab, Pakistan. The morphological and anatomical descriptions and molecular analysis of the *L. birnbaumii* provides a baseline data for future taxonomic work on this species and related taxa which will be helpful in various applied fields particularly pharmaceutical industry.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Hira Ijaz: Drafted the manuscript and helped in phylogenetic analysis

Maham Mureed: Collected the sample and worked on it for her thesis

Aneela Yasmeen: Helped in molecular work and formatted the document

Arooj Naseer: Helped in microscopy

Abrar Hussain: Supervisor of Maham Mureed, reviewed the document.

Sana Jabeen: Co Supervisor of Maham Mureed, took responsibility of the document, reviewed it, made plates and did phylogenetic analysis.

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