

## *Lentinus squarrosulus* an edible macro-fungus reported from Pakistan

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### Abstract

*Lentinus squarrosulus* (Mont.) Singer: an edible macro-fungus is reported from the moist temperate Himalayan forests of Pakistan. The sample was studied based on morphological characteristics and nucleotide sequence analyses of the ITS region. Studies based on morpho-anatomical and molecular data are consistent with formerly reported specimens. *Lentinus squarrosulus* represents the first report for Pakistan.

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### INTRODUCTION

Genus *Lentinus* (Fr.) Quel' belongs to the class Agaricomycetes and family Polyporaceae and is recognized by about 63 species from over the world<sup>[1,2]</sup>, while Index Fungorum ([www.indexfungorum.org](http://www.indexfungorum.org)) lists 636 records of *Lentinus*. Commonly *Lentinus* species are wood-decaying basidiomycetes. Morphological characteristic of these species is decurrent lamellae while anatomical features are dimitic tissues, hyaline, ellipsoid to cylindric spores. Most of the basidiomes have a long life span and are found in dry conditions due which gives them a tough texture upon drying. Species of this genus has different habitats and are frequently found in tropic and temperate zones<sup>[2]</sup>. Almost all the members of *Lentinus* are edible except those which have a tough texture. Therefore, it is important to commercialize the edible species of this genus. *Lentinus squarrosulus* is a wild edible mushroom that is commonly found growing on decaying logs of trees during the rainy season. Similar to other species of macrofungi, this mushroom can grow on a wide variety of substrates and habitats. Many species of *Lentinus* have been reported to grow naturally on special substrates and can be grown on pasteurized substrates<sup>[3,4]</sup>.

Only four species of *Lentinus* have been reported from Pakistan<sup>[5]</sup>. A large myco-diversity is found in the northern areas of Pakistan, especially district Buner is one of the lush green areas located in the northern zone of Pakistan. The richness of wild mushrooms is also one bio-indicator of ecosystem health. Species identification based on morphological methods is not reliable, as some poisonous species are confused with edible species<sup>[6–8]</sup>. Recently, several individuals have lost their lives mistakenly eating poisonous mushrooms which are morphologically similar to edible mushrooms<sup>[8]</sup>.

There is a high myco-diversity found in this area based on the availability of litter and favorable climatic conditions. Most of the people of this area collect the wild mushrooms for food purposes but unfortunately, it is totally unexplored and only

four species have been reported<sup>[8]</sup>. In the present investigation, an edible macro-fungus *Lentinus squarrosulus* is reported as a new record for Pakistan based on morphological and molecular studies.

### MATERIALS AND METHODS

#### Sampling sites

Specimens were collected in the mountain region of Gokand Valley, Buner District, Khyber Pakhtunkhwa, Pakistan during the rainy seasons of 2017–2018. Specimens were tagged and photographed in the field and morphological features were noted from fresh basidiomes. The specimens were then dried with the help of a fan heater and kept in small zip-lock bags for further analysis. The collection was vouchered and deposited to the Herbarium of the Department of Botany, Hazara University Mansehra, Pakistan (HUP-11565).

#### Morphological observations

Morphological features of the fresh basidiomes were used for color codes<sup>[9]</sup>. Morphological features comprised pileus size, shape, and color; color of gills and their mode of attachment to stipe; stipe color and their attachment to pileus; presence or absence of volva and annulus. For microscopical studies, the pileal, gill and stipe tissues were mounted in 5% KOH and stained with 1% (w/v) aqueous Congo red and observed under a Labomed light microscope. For each specimen, 25 measurements of length and width in basidiospores, basidia, and cystidia were taken. Basidiospore dimensions include length and width ranges (with parenthetical extreme values), range of Q coefficients (L/W), mean length and width (Lm × Wm), and mean Q value (Qm).

#### DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from the small portion of pileus using the CTAB method<sup>[10]</sup> with some modifications. Internal transcribed spacer (ITS) of the nuclear ribosomal DNA

(rDNA) was amplified by using the fungal-specific ITS1F (5-cttggtcattagaggaagtaa-3) and ITS4 (5-tcctccgcttattgatgc-3)[11]. The amplified products were sent to BGI Shenzhen, China, for purification and sequencing.

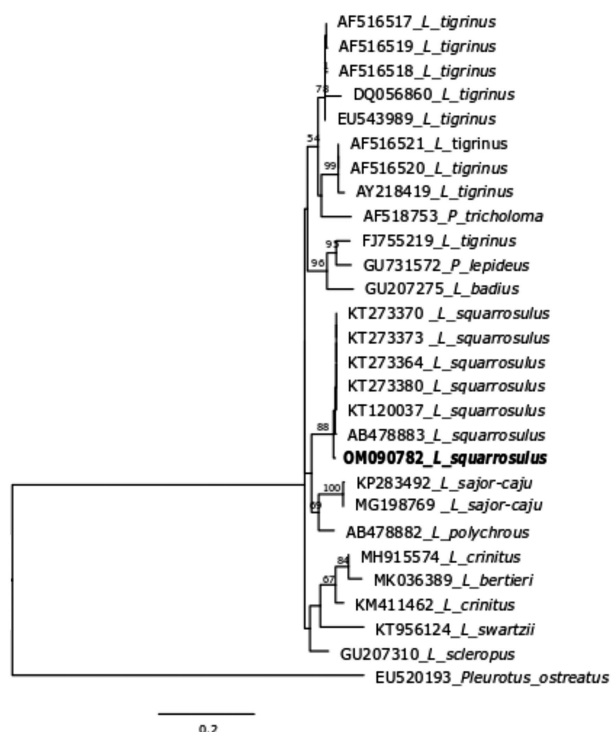
### Phylogenetic analyses

ITS sequences were BLAST searched at NCBI GenBank database and closely matched sequences from GenBank were downloaded for phylogenetic analyses based on BLAST analysis and sequences of the same section from published articles[2]. All the sequences were aligned in BioEdit[12] and trimmed to facilitate alignment, while sequence alignments were done with MAFFT v.6.864b and alignments were manually improved if necessary, using BioEdit. A maximum-likelihood tree was inferred for the ITS alignment using RAXMLHPC2v 8.1.11[13] with a GTRCAT nucleotide substitution model. Rapid bootstrapping was performed with 1000 bootstrap iterations. All phylogenetic analyses were performed on the CIPRES Science Gateway v3.1[14]. The phylogeny from ML analysis (Fig. 1) was displayed with FigTree v1.4.2 ([tree.bio.ed.ac.uk/software/figtree](http://tree.bio.ed.ac.uk/software/figtree)) and then edited in Adobe Illustrator. The newly generated sequences were deposited in GenBank.

## RESULTS

### Phylogenetic analyses Fig. 1

For sequencing of *Lentinus squarrosulus*, PCR products using ITS primer yielded 643–706 base pairs. BLAST of the consensus sequence of 458 bp showed 99% similarities with *L. squarrosulus* from Nigeria (KT120037, KT273380 & KT273373) and Kenya (KT273370, KT273364) 100% query cover and 0.0 E value.

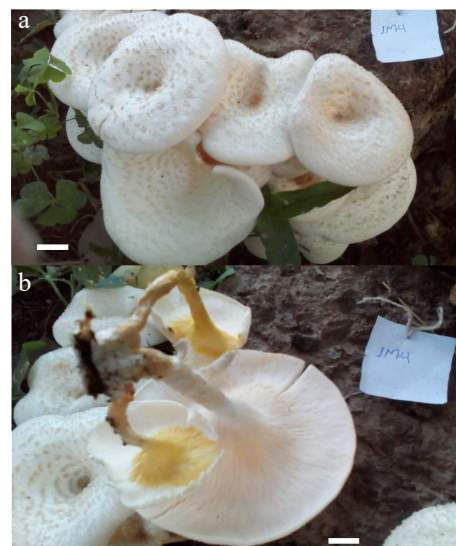


**Fig. 1** Phylogenetic tree of *Lentinus* and *Polyporus* genera. RAXML tree based on ITS phylogeny of *Lentinus* and related genera. *Pleurotus ostreatus* was selected as an outgroup. The Pakistani species is indicated in bold.

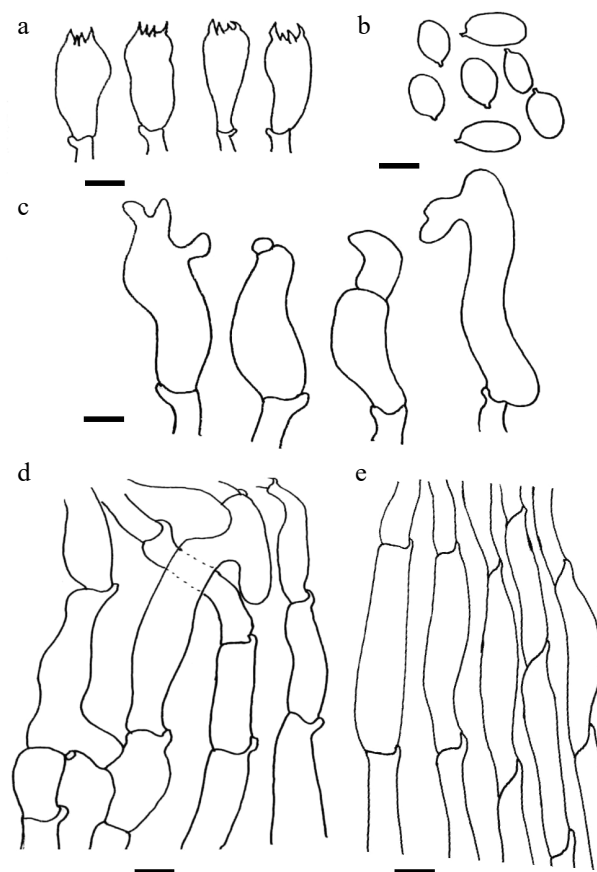
### Taxonomic description

*Lentinus squarrosulus* Mont., Annls Sci. Nat., Bot., ser. 2., 18: 21 (1842) Figs 2 and 3

Genbank Number: OM090782



**Fig. 2** Morphological features of *Lentinus squarrosulus* HUP-11565. (a) Pileal view with squarrose scales. (b) Lamellae. Scale bars = 10 mm.



**Fig. 3** Anatomical features of *Lentinus squarrosulus*. (a) Basidia. (b) Basidiospores. (c) Cheiliocystidia. (d) Pileipellis. (e) Stipitipellis. Scale bars: (a) 3 μm; (b) 2 μm; (c) 4 μm; (d) 3 μm; (e) 5 μm.

## Lentinus squarrosulus

*Pileus*: 30–40 mm in diam, funnel-shaped, initially white (2.5Y9/2) become pale brown (10YR 8/3) at maturity, entire with varying pale brownish (10YR 8/3) concentric zones of light yellowish brown (10YR 8/3) squarrose scales on the surface, margin smooth, incurved. *Lamellae*: decurrent, close to crowded, unequal, smooth edges, white (2.5Y 9/2) to pale brown color (10YR 8/3). *Stipe*: 20–40 × 5–10 mm, white (2.5Y 9/2) to light-brown (10YR 8/3), smooth, cylindrical to slightly broad at the base.

*Basidiospores* (3.6–) 3.8–4.8 (–5) × (1.4–) 1.7–2.2 (–2.3) µm [L × W = 4.2 × 2 µm, Q = (1.8) 1.9–2.5 (2.9), Q<sub>e</sub> = 2.2], subglobose to subcylindrical, smooth, thin-walled, hyaline. *Basidia*: 11–13.5 × 2.3–3.8 µm, tetra-spore, clavate, thin-walled. *Cheilocystidia* 15–33 × 7–8.2 µm, narrowly clavate to utriform have small outgrowth, thin-walled. *Pileipellis* hyphae 3.2–4.7 µm wide, branched. *Stipitipellis* hyphae 4.1–6.2 µm wide, septate. *Clamp connections*: present.

Specimen examined: Pakistan, Khyber Pakhtunkhwa Province, Buner District, Gokand Valley 1200 m asl, on a rotten trunk of *Populus nigra* L. August 16, 2017, M. Ishaq (IM-4).

## DISCUSSION

The genus *Lentinus* consists of wood-decaying species that are frequently edible and widely used for food as well as for therapeutic purposes. In India, *Lentinus* is becoming one of a potential edible mushroom containing many vitamins and having nutraceuticals, antimicrobial, and antioxidant properties<sup>[15]</sup>. It has high nutritional value due to the presence of abundant proteins, amino acids, sugars, vitamin B, C, and D, minerals, and lipids<sup>[4]</sup>.

*Lentinus* species are commonly known as white rot fungi and can be saprobic fungi at all-time phases. *Lentinus squarrosulus* is an edible species and cosmopolitan in nature. It is very difficult to prepare its spawn, but some researchers have been successful to culture and domesticate them. This is the reason that it has not been cultivated on a large scale, however, it is very popular as an edible mushroom in central Africa<sup>[15]</sup>. It is reported in Thailand, South-East Asia, the Southern part of Nigeria, the equatorial region of Africa, the Pacific islands, Northern, Southern India, Australia, Philippines, and Indonesia<sup>[16–18]</sup>. This is the first report of this species from Pakistan.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Dates

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