

Panus conchatus; A new record (lentinioid fungi, Basidiomycota) from Pakistan

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ABSTRACT

An unrecorded *Panus* species was collected during the field survey in Malakand district, Khyber Pakhtunkhwa, Pakistan. Based on morphological characteristics and molecular data from internal transcribed spacer rDNA sequence analyses, the species was identified as *Panus conchatus*. Literature review confirms this to be the first record of the species from Pakistan.

Key words— basidiomycetes, lentinioid fungi, polypore, taxonomy, molecular data

INTRODUCTION

The genus *Panus* Fr. is placed within the residual polyporoid clade (Binder *et al.* 2013). The 231 taxa of *Panus* are listed in Index Fungorum (Accessed date: 15 August 2019) and 206 taxa are listed in MycoBank (MycoBank 2019; accessed date: 15 August 2019). *Panus* species are regarded as white rot fungi (Zhou *et al.* 2016), and are usually widely distributed in sub-tropical to tropical, temperate, and boreal regions in proximity to a wide range of broadleaf trees (Pegler 1975, 1983, Corner 1981, Vargas-Isla *et al.* 2015). Two *Panus* species in particular, *P. conchatus* and *P. lecomtei*, are distributed in tropical areas and irradiated in temperate regions (Zmitrovich *et al.* 2018).

They are commonly occurring in the tropics and less so in north temperate regions (singer 1986). Fries (1936-1838) describe *Panus conchatus* (Bull, Fr.) Fr., as the type species of this genus, it is an inedible species of mushroom that occurs throughout the Northern Hemisphere. The fungus grows on the decomposing wood of a wide variety of deciduous and coniferous trees. The specific epithet *conchatus* is derived from the Latin meaning "shell-like". It is commonly known as the lilac oysterling. *Panus conchatus* mushrooms have an extremely variable morphology that changes with the age of the fruit bodies. According to him the basidiocarps are pleurotoid, tough, cartilaginous, and revive upon moistening. The stipe is central to lateral or sometimes almost absent. The pileus surface is tomentose, strigose, squamulose or smooth, and the gills are decurrent with an entire margin. Basidiocarps are usually not brightly colored except for those taxa which exhibit a lilac pigment. The context is white and composed of interwoven hyphae which become dimittic in age, exhibiting intercalary or terminal skeletal hyphae. The basidia are clavate and four sterigmata. The basidiospores are white, ellipsoid to cylindric, smooth, thin-walled, and inamyloid. Cystidia are present in some taxa. Corners (1981) include those taxa with a dimittic hyphal system, cylindric basidiospores, pleurocystidia in most taxa, and the absence of hyphal pegs and have an irregular lamellar trama in *Panus*. Despite being a gilled species, phylogenetic analysis has shown it is closely related to the pored species found in the Polyporaceae. By Using rDNA, Hibbett and Vilgalys (1991) supported the conclusion about the data of genus *Panus* by corner (1981) and singer (1986). Mushrooms in the genus *Panus* are regarded as free gilled and form a monophyletic clade with *Lentinus* and *Polyporus* (Hussein *et al.* 2014).

In Pakistan, previously four *Panus* species have been reported viz: *Panus Crinitus* (L. ex FR.) Singer, *P. Rudis* Fr. and *P. Tigrinus* (Bull. ex Fr.) Singer. By (Ahmad 1980) and *P. Stypticus* (Bull. ex Fr.) Karst. was reported by Iqbal and Khalid (1996). During our studies on diversity of polyporoid fungi in KP Forests, an unreported *Panus* specie was found. The basidiomata were first identified using morphological characters, and sequence analyses of internal transcribed spacer (ITS) rDNA region supported the identification. A detailed description of the Pakistani *Panus* species is provided below.

MATERIALS & METHODS

Collections

Collections of the basidiocarps are based on routine field visits to the forests of Malakand district, during 2015. Important macroscopic features were noted from fresh specimens using a field notebook. The specimens were dried using a fan heater with temperature adjusted at 40°C.

Microscopic observation

Macro and microscopic basidiocarp features were noted from the collected specimens. Measurements and drawings were made from slide preparations mounted in a drop of lactic acid or Melzer's reagent or 5% KOH on slides and were observed (Largent *et al.* 1977) Anatomical features were measured using calibrated Piximetre software connected to a B-350 Optika compound microscope through a Scope image (H9D) microscopic camera and visualized on a computer screen.

Twenty basidiospores, basidia, and cystidia were measured; Q = the length/width ratio of a single spore; Qe = average length/width ratio of all spores; Me = average L × W of all spores measured.

DNA extraction, amplification, sequencing, and molecular phylogenetic analysis

Molecular Characterization

About 50 mg of dried gills were ground into fine powder using liquid nitrogen, and the DNA was extracted according to Gardes & Bruns (1996). The universal primer pair ITS1F and ITS 4 (White *et al.* 1990, Gardes & Bruns 1993) was used to amplify the rDNA ITS (ITS1F+5.8s+ITS4) region in 50 µL reaction volumes following Gardes & Bruns (1993). The PCR amplified product was sequenced by Beijing Genomic Institute (Hong Kong). BLAST analysis was performed using the National Center for Biotechnology Information (USA) database, and closely matching sequences were downloaded for further phylogenetic analysis. Sequences were aligned using MEGA 7 software (Kumar *et al.* 2016). Sequences are deposited in GenBank.

RESULTS

Molecular phylogeny

Fig. 1

Amplification of using the ITS1F and ITS4 primers generated a 650bp sequence. The initial BLAST comparison of the Pakistani sequences showed 99% identity and 94% query cover with *Panus conchatus* (Bull.) Fr., KP776992.1 and 98% identity and 94% query cover with KR818817.1.

Identical sequences were retrieved from GENBANK and were aligned through online tool muscle. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. The MEGA7 maximum likelihood analysis clustered our Pakistan sequences with the *P. conchatus* sequences in a separate clade with a strong bootstrap value of 100%, thereby supporting a new record of Pakistani Species *P. conchatus* phylogenetically as the same species of *P. conchatus* present in GENBANK.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. The percentage of trees in which the associated taxa clustered together is

shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Estimations were done by employing the MCL (maximum composite likelihood) criteria. Subsequently the topology with uppermost log likelihood value was selected. Number of substitutions per site was used for scaling the tree. Representation of the evolutionary history of the taxa analyzed was shown by inferring the bootstrap consensus tree inferred from 1000 replicates is shown. Final dataset had 454 characters exclusive. Among these 404/615 were conserved characters, 211 were variable, 172 were parsimony informative and 39 were singletons. The analysis was carried out for 43 representative sequences. Both coding and non-coding positions were included in the analysis. Those positions in which data was missing were eliminated. The analysis involved 23 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 336 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Fig. 3.45).

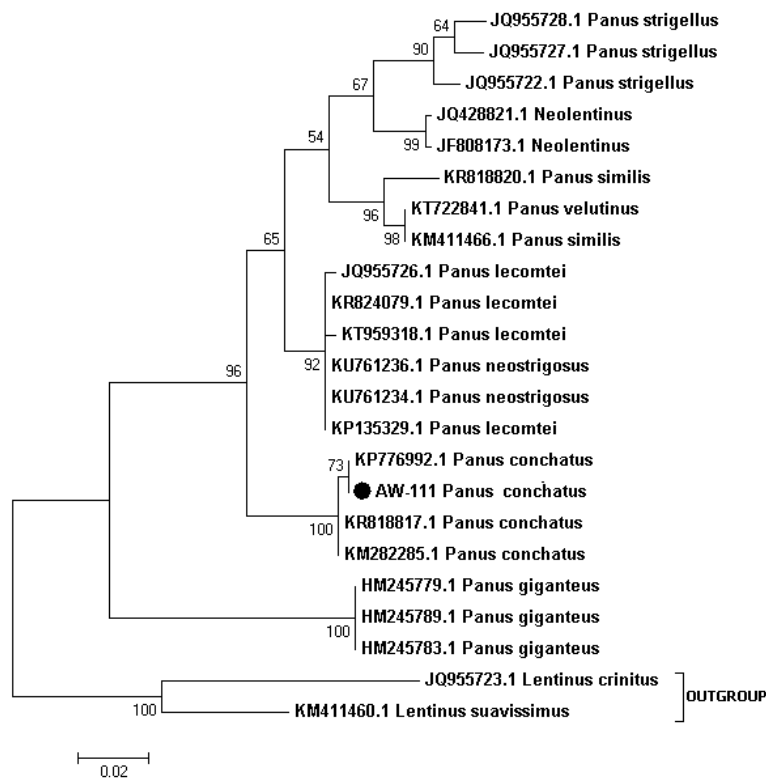


Figure-1. Molecular phylogenetic analysis of *Panus Conchatus* by the Maximum Likelihood method. The tree with the highest log likelihood (-1428.1482) is shown. Collection of Malakand is represented by black dot (●). Species of *Lentinus* were used as outgroup.

TAXONOMY

Panus Conchatus (Bull.) Fr., *Epicr. syst. mycol.* (Upsaliae): 396 (1838) [1836-1838] Plate 1-Figs. A-H&1

Morpho-anatomical characterization

Basidiocarp: Pileus 2-3cm broad, spathulate to conchate in profile, becoming depressed in the center at age; margin decurved, wavy; surface glabrous, dry, appearing radially striate to fibrilose scaly, scales more apparent in mature basidiocarps, appressed in age; light brown to brown, scales and fibrils brown to dark brown. Lamellae decurrent, close, broad, margin entire, forked, lamellulae in 3-4 tiers; white to cream. Stipe central, eccentric or lateral, 1-2 cm long, 0.5-1cm in diameter, equal or tapering to the base; surface fibrilose to pubescent, dry; light brown to reddish grey. Context white to cream, not staining, up to 1 cm thick over the surface of the pileus. Basidiospores $Q = 1.8 - 1.89 (1.9)$; $X = 6 \times 3.3 \mu\text{m}$; $Q_e = 1.8$, ellipsoid to cylindric, hyaline, thin-walled, inamyloid, white to cream colored in mass. Basidia $Q = (3.2) 3.5 - 4.6 (4.9)$; $X = 18 \times 4.7 \mu\text{m}$; $Q_e = 3.9$, four sterigmate, clavate, thin-walled, hyaline, clamped. Hyphal system: Trama comprised of interwoven generative and skeletal hyphae. Generative hyphae 2-4.5 μm in diameter, filamentous branched, thin-walled, hyaline, clamped. Skeletal hyphae 3-6.5 μm in diameter, filamentous unbranched, walls up to 2 μm thick, hyaline, somewhat refractive, intercalary or terminal in origin. Pileipellis a simple cutis of radially arranged, repent, generative hyphae, 2-4.5 μm in diameter, filamentous branched, walls brown, slightly thickened, clamped.

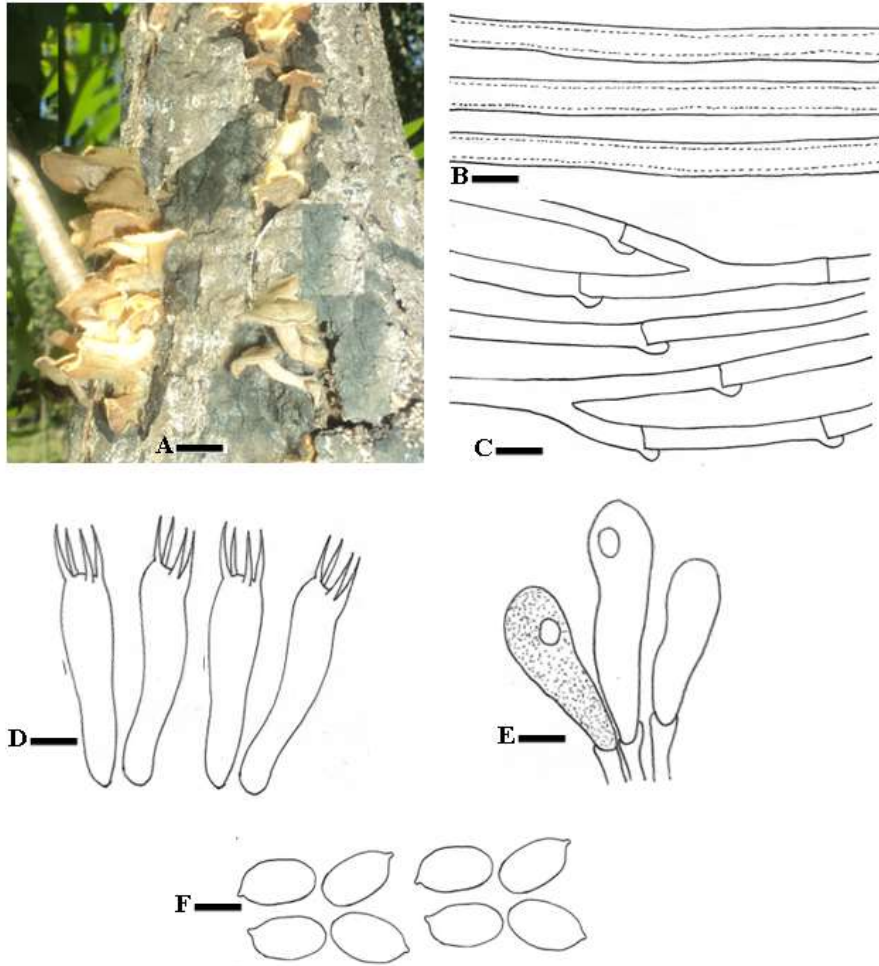
Habit: gregarious on hardwoods.

Habitat: On stem bark of *Melia azedarach*.

Specimens examined: Pakistan, Khyber Pakhtunkhwa, Malakand, Totakan, 513 m asl, on bark of *Melia azedarach*. 08. 8 2015, A. Wahab (AW-105).

Remarks — this species is recognized by spathulate to conchate in profile, becoming depressed in the center at age; margin decurved, wavy; surface glabrous, dry, appearing radially striate to fibrilose scaly, scales more apparent in mature basidiocarps.

Plate 1



Figures (A-F) Morpho-anatomical shapes of *Panus Conchatus* (A) Basidiocarp. (B) Skeletal hyphae (C). Generative hyphae (D) Basidia. (E) Cystidia (F) Basidiospores. Scale bar A=2-3cm, B=2.5 μ m, C=2 μ m, D=2.3 μ m, E=2 μ m, F=3.4 μ m.

Microscopic features:

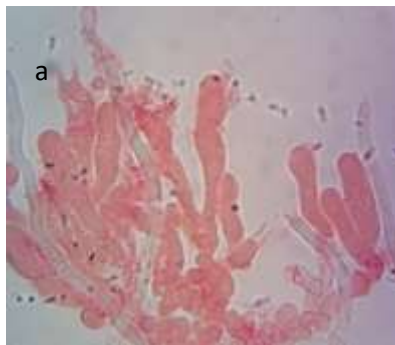


Figure- a. basidia,



b.basidiospore

DISCUSSION

Previously four species *Panus Crinitus* (L. ex FR.) Singer, *P. Rudis* Fr., *P. Tigrinus* (Bull. ex Fr.) Singer. And *P. Stypticus* (Bull. Ex Fr.) Karst. have been reported from Pakistan (Ahmad 1980; Iqbal and Khalid (1996). During our study on diversity of polyporoid fungi in KP Forests, an unreported *Panus* species was found on tree trunk of *Melia azedarach*. And the morphological and Molecular analysis clusters the present Pakistani collection with *P. Conchatus*.

The genus *Panus* Fr. is placed within the residual Polyporoid clade (Binder *et al.* 2013). They are commonly occurring in the tropics and some in north temperate regions (singer 1986). Fries (1836-1838) describe *Panus conchatus* (Bull.) Fr., as the type species of this genus, it is an inedible species that occurs throughout the Northern Hemisphere. The fungus grows on the decomposing wood of a wide variety of deciduous and coniferous trees. The specific epithet *conchatus* is derived from the Latin meaning "shell-like". It is commonly known as the lilac oysterling. *Panus conchatus* have an extremely variable morphology that changes with the age of the fruit bodies. Previously four species of *Panus*, viz: *Panus Crinitus* (L. ex FR.) Singer, *P. Rudis* Fr., *P. Tigrinus* (Bull. ex Fr.) Singer. and *P. Stypticus* (Bull. ex Fr.) Karst. was reported from Pakistan (Ahmad 1980, Iqbal and Khalid 1996).

During our study on diversity of Polyporoid fungi *Panus conchatus* is found as new record in KP Forests. It is characterized by Pleurotoid basidiocarps, tough, cartilaginous, and revive upon moistening. The pileus surface is tomentose, strigose, and the gills are decurrent with an entire margin with central stipe. Basidiocarps are usually not brightly colored except for those taxa which exhibit a lilac pigment. The context is white and composed of interwoven dimitic hyphae, exhibiting intercalary or terminal skeletal hyphae. The size of Basidiospores is $6 \times 3.3 \mu\text{m}$ in diameter, ellipsoid to cylindric, hyaline, thin-walled, inamyloid, white to cream colored in mass. Corner (1981) include those taxa in *Panus* with a dimitic hyphal system, cylindric basidiospores, pleurocystidia, have an irregular lamellar trama and the absence of hyphal pegs. The initial BLAST comparison of the this collection showed 99% maximum identity with *Panus conchatus* (Bull.) Fr., (GENBANK KP776992.1 and KR818817.1). Despite being a gilled species, phylogenetic analysis has shown it is closely related to the pored species found in the Polyporaceae. Hibbett and Vilgalys (1991) use rDNA regions to support the conclusion about the data of genus *Panus* by Corner (1981) and Singer (1986). The current phylogenetic analysis clustered our Pakistan sequences with the *P. conchatus* sequence in a separate clade with 100% bootstrap value of (Fig. 1) thereby supporting a new record of Pakistan which is phylogenetically similar to species of *P. conchatus* present in GENBANK.

However, information regarding this species is still limited and the abilities of this species should be investigated further.

CONCLUSIONS

The ITS rDNA phylogenetic trees had similar topologies with panus species forming a separate clade with *P. chonchatus* (Fig. 1). On the other hand Mushrooms in the genus *Panus* are regarded as free gilled and form a monophyletic clade with *Lentinus* and *Polyporus* (Hussein *et al.* 2014). However, information regarding this species is still limited and the abilities of this species should be investigated further.

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