



## Morphological and molecular identification of *Panus conchatus* (Polyporaceae, Polyporales) from Yunnan Province, China

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### Abstract

*Panus conchatus* is a species of lentinioid fungi in the family Polyporaceae. This species is characterized by its concave, smooth, deeply decurrent gills, with distinctive purple grey to greyish magenta basidiocarps. This fungus is widely distributed in both tropical and temperate regions. Here, we report a specimen of *P. conchatus* collected from a temperate region in Yunnan Province, China. The specimen is described and illustrated based on macro- and micro-morphological characteristics. Phylogenetic analyses were done based on the sequence data of ITS and LSU, and the placement of the taxon was confirmed. This is the first time *P. conchatus* has been reported with molecular phylogenetic data from China. Full description, illustrations, color photographs, and a phylogenetic tree to show the placement of *P. conchatus* are provided.

**Keywords** – agaricoid form – hymenophoral trama – lentinioid mushroom – polyporales

### Introduction

Mushrooms in the genus *Panus* are regarded as free gilled and form a monophyletic clade with *Lentinus* and *Polyporus* (Hussein et al. 2014). The genus *Panus* Fr. was introduced by Fries (1838), with *P. conchatus* as the type species (Fries 1838). *Panus* is an agaricoid genus that was introduced as a distinct lentinioid fungus in the order Polyporales (Senthilarasu 2014) and is considered as a subgenus of *Lentinus* (Pegler 1983). *Panus* species are characterized by free gills; strongly radiate hymenophoral trama; somewhat leathery, often dimitic hyphae; abundant skeletal hyphae (typically unbranched); and a lack of hyphal pegs (Hard 1908, Hibbett et al. 1993, Hussein et al. 2014, Senthilarasu 2014).

Various authors have also identified different *Panus* and *Lentinus* morphological characteristics (Corner 1981, Thorn et al. 2000, Moncalvo et al. 2000, Drechsler-Santos et al. 2012). However, *Panus* is clearly distinguished by the presence of the hyphal system, in particular the thick-walled skeletal hyphae with unbranched or ligative hyphae in the context (Corner 1981), and its gills are also more thin and smooth edged than *Lentinus* (Hard 1908).

*Panus* is regarded inappropriately as a food source (Vargas-Isla et al. 2015), while some *Panus* species, such as *P. conchatus*, *P. crinitus*, *P. lecomtei*, *P. rudis*, and *P. strigellus* are generally regarded as edible mushrooms by people in Brazil, Colombia and other countries in South America (Hard 1908, Fidalgo & Prance 1976, Vargas-Isla et al. 2015). *Panus* is also a laccase fungal group, and contains a wide range of bioactive compounds as well as panepoxydone, panutorulon A, naematolin, and naematolon (Zjawion 2004, Zaidman et al. 2005, Ding et al. 2018). These compounds are used in natural remedies to cure various pathological diseases, such as cancers and cardiovascular diseases, and they possess hepatoprotective, antibacterial, antioxidant, and antiviral properties (Soares et al. 2013). *Panus* are used in a wide range of applications, including as white laccate fungi for pulp bleaching; in water treatment; and in the removal of phenols in the food industry (Zhou et al. 2014). The panepoxydone derived from *P. conchatus* and *P. rudis* is also known to inhibit NF- $\kappa$ B-mediated signal transduction in animal cells (Zjawion 2004).

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).

*Panus conchatus* (Bull.) Fr. was introduced as *Agaricus conchatus* Bull. based on laterally, smooth, and usually pale purple to purple-brown upper surface to the entire margin (Senthilarasu 2014). This fungus has been reported as a common species distributed worldwide (Senthilarasu 2014, Zmitrovich et al. 2018). Ding et al. (2018) reported *P. conchatus* to be distributed in Henan, Jilin, Shaanxi, and Yunnan, China, but the findings were published without taxonomic and phylogenetic evidence. In this study, we introduce *P. conchatus* from Yunnan Province, China with color photographs, macro- and micro-morphological descriptions, and a phylogenetic tree to show the placement of the taxon.

## Material and Methods

### Sample collection and isolation

The basidiocarps were collected from a logwood of *Pinus kesiya* (Royle.) at the Kunming Institute of Botany, Yunnan Province, China, in December 2017. The internal tissues of the basidiocarps were placed on potato dextrose agar (PDA) under aseptic conditions to get a pure culture and incubated at 25°C for 14 days (Luangharn et al. 2017). Pure culture was deposited in the culture collection of the Kunming Institute of Botany (KUMCC) with the voucher number KUMCC18-0047. The cultures were maintained at 4°C for further study. The specimens were hot air dried at 40°C for 2 days and covered with wax paper containing dehydrated silica gel as a desiccant to control humidity. The herbarium specimens were deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica (HKAS), Chinese Academy of Science, Yunnan Province, China, with the voucher number HKAS 97487.

### Morphological study

Macro-morphological characteristics were obtained from fresh specimens following the methods previously described by Lodge et al. (2004) and Largent (1986). Color codes were recorded following (Ridgeway 1912). Microscopic characteristics were observed under an OLYMPUS SZ61 stereo microscope, with dried materials rehydrated in Congo red and 5% KOH. Micro-morphological characteristics were observed under a Nikon ECLIPSE Ni (Nikon, Tokyo, Japan) compound microscope objective lenses of 10 $\times$ , 20 $\times$ , 40 $\times$  and 100 $\times$ . Photographs were taken with a Canon EOS 600D (Tokyo, Japan) digital camera fitted to the microscope. Measurements were taken using Tarosoft<sup>®</sup> Image Framework program v. 0.9.0.7. The size and shape of

basidiospores followed Miettinen & Larsson (2006) with a minimum of 50 basidiospore measurements from each basidiocarp. The photographs and drawing plates were edited in Adobe Photoshop CS3.

### **DNA extraction, polymerase chain reaction (PCR), and sequencing**

Fungal mycelia were grown on PDA media; after incubation at 25°C for 2 weeks, fungal mycelia were scraped off and transferred into 1.5 ml sterile tubes for DNA extraction. The Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) was used to extract DNA, following the manufacturer's instructions. PCR amplifications were performed in a total volume of 25 µL of PCR mixtures containing 9.5 µL of ddH<sub>2</sub>O, 12.5 µL of PCR master mix, 1 µL of DNA sample, and 1 µL of each primer. PCR amplification was carried out using primers LROR/LR5 for the nuclear ribosomal large subunit 28S rDNA gene (LSU) (Vilgalys & Hester 1990), and ITS5/ITS4 for the internal transcribed spacer rDNA region (ITS1, 5.8S rDNA and ITS2) (White et al. 1990). PCR amplification for ITS was performed as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles at 95°C for 30 s, 55°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 10 min. PCR amplification for LSU was performed as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 35 s, 55°C for 50 s, 72°C for 1 min, and a final extension of 72°C for 10 min. The sequencing of PCR products was carried out by Sangon Biotech (Shanghai) Co. Ltd. in China. The nucleotide sequence data acquired was deposited in GenBank to obtain the accession numbers.

### **Phylogenetic analyses**

A total of 36 taxa included in the phylogenetic analyses retrieved from GenBank based on recent publications are shown in Table 1. Sequences of closely related strains were retrieved based on BLAST search results in GenBank (<http://www.ncbi.nlm.nih.gov>). Single sequence alignment was generated with online sequence alignment tools MAFFT v. 7.394 (<https://mafft.cbrc.jp/alignment/software/>) (Kato & Standley 2013) and manually edited in BioEdit v. 7.0.9 (Hall 1999) and Clustal X (Thompson et al. 1997). To confirm the phylogenetic positions of fungal species, sequences of ITS and LSU were combined and analyzed with Maximum likelihood (ML), Maximum parsimony (MP), and Bayesian inference posterior probabilities (PP). The alignments were checked visually and improved manually where necessary, and gaps were treated as missing data.

Maximum likelihood phylogenetic analysis was performed using RAxML v. 7.2.6 (Stamatakis 2006) on the RAxML-HPC2 on XSEDE (v. 8.2.8) of the CIPRES science Gateway (<https://www.phylo.org>) (Miller et al. 2010), and a duplication was carried out using raxmlGUI v.0.9b2 (Silvestro & Michalak 2010) with default parameters and 1000 bootstrap replicates. The final tree was selected from among suboptimal trees by comparing likelihood scores under the GTRGAMMA substitution model.

For Bayesian Inference (BI) analyses, best models of evolution were estimated by using MrModeltest 2.3 (Nylander 2004), and the Bayesian posterior probabilities (PP) were estimated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.2 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1 million generations and trees were sampled every 100 generations; thus 10,000 trees were obtained. The suitable burn-in phases were determined by traces inspected in Tracer version 1.6 (Rambaut et al. 2014). Based on the tracer analysis, the first 2,000 trees, representing 20 % of the burn-in phase of the analyses, were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree. Maximum parsimony (MP) analyses were made using PAUP v. 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search with random addition of sequences with 1000 replicates.

The resulting trees and data files were viewed in FigTree v. 1.4 program (Rambaut & Drummond 2008) and edited using Microsoft Office PowerPoint 2010 and Adobe Illustrator CS3 (Adobe Systems Inc., USA). MLBP and MPBP equal to or greater than 70%, and PP equal to or

greater than 0.90 on the nodes are considered as significantly supported. Since the topologies of ML, MP and Bayesian analyses are the same, the ML tree was selected to show the placement of the taxon.

**Table 1** Taxa and their GenBank accession numbers used in the phylogenetic analyses of this study.

Fungal species	Voucher	GenBank accession no.		References
		ITS	LSU	
<i>Panus conchatus</i>	KUMCC 18-0047	MK192053	MK333258	This study
<i>P. conchatus</i>	X1234	JN710579	-	Miettinen et al. 2012
<i>P. conchatus</i>	FLAS-F-60901	MH016880	-	GenBank
<i>P. conchatus</i>	CLZhao 1535	MG231759	-	GenBank
<i>P. conchatus</i>	4314	-	AY616003	Hussein et al. 2014
<i>P. conchatus</i>	6254	-	AY616004	Hussein et al. 2014
<i>P. conchatus</i>	LE265028	KM41146	KM434323	Zmitrovich & Kovalenko 2016
<i>P. conchatus</i>	JMH44	KM267730	-	Hussein et al. 2014
<i>P. fulvus</i>	LCF573	-	AY615997	GenBank
<i>P. fulvus</i>	10689	-	AY615996	GenBank
<i>P. lecomtei</i>	TMIC35103	JQ955726	-	Vargas-Isla et al. 2015
<i>P. lecomtei</i>	HHB-11042-Sp	KP135328	KP135233	Floudas & Hibbett 2015
<i>P. lecomtei</i>	HHB-9614	KP135329	-	Floudas & Hibbett 2015
<i>P. neostrigosus</i>	LSPQ-NSM-106	KU761234	KU761114	Dufresne et al. 2017
<i>P. neostrigosus</i>	LSPQ-NSM-107	KU761235	KU761115	Dufresne et al. 2017
<i>P. neostrigosus</i>	LSPQ-NSM-108	KU761236	KU761118	Dufresne et al. 2017
<i>P. rudis</i>	ZJ1005DKJ02	KU863049	-	GenBank
<i>P. rudis</i>	ZJ1005DKJ03	KU863050	-	GenBank
<i>P. rudis</i>	ZJ1005DKJ04	KU863051	-	GenBank
<i>P. similis</i>	KWGM 39	KY630517	-	GenBank
<i>P. similis</i>	DMC 189	-	EU908182	Douanla-Meli & Langer 2010
<i>P. similis</i>	LE287548	KM411466	KM411482	Zmitrovich & Kovalenko 2016
<i>P. strigellus</i>	9114	-	AY616001	GenBank
<i>P. strigellus</i>	INPA239979	JQ955724	JQ955731	GenBank
<i>P. strigellus</i>	TENN55993	JQ955728	-	GenBank
<i>P. strigellus</i>	TENN56192	JQ955727	-	GenBank
<i>P. strigellus</i>	INPA222827	JQ955722	-	GenBank
<i>P. velutinus</i>	NAL318	-	GQ487335	Douanla-Meli & Langer 2010
<i>P. velutinus</i>	DMC 694	-	EU908187	Douanla-Meli & Langer 2010
<i>P. velutinus</i>	DMC 695	-	EU908188	Douanla-Meli & Langer 2010
<i>Panus</i> sp.	M85	KP096364	-	GenBank
<i>Panus</i> sp.	MEL 2382698	KP012877	-	GenBank
<i>Panus</i> sp.	MEL 2382967	KP012827	-	GenBank
<i>Polyporus melanopus</i>	CIEFAP148	AF516569	-	GenBank
<i>P. conifericola</i>	Cui9950	KU189783	-	Zhou et al. 2016

## Results

### Phylogenetic analyses

The sequence dataset comprises 23 ITS and 17 LSU from 35 samples and includes 33 ingroup taxa and 2 outgroup taxa (*Polyporus melanopus* CIEFAP 148 and *P. conifericola* Cui 9950) (Table 1). The dataset had an aligned length of 1,487 total characters, of which 1,164 were constant, while 246 variable characters were parsimony-informative, and 77 characters were parsimony-uninformative. The ML analyses carried out from RAxML and raxmlGUI analyses

provided similar results, and the topology of the ML tree was similar to that of the PP tree. Here, only RAxML ML tree topologies were chosen, and they are shown in Figure 1. Nine *Panus* species used in this study were grouped together MLBS = 100%/ MPBS = 100%/ PP = 1.00, and segregated into two main clades. Tree topologies of our study were similar to that of Hussein et al. (2014). In the phylogenetic tree, seven clades were seen, of which six were *Panus* species; including *P. conchatus*, *P. fulvus*, *P. lecomtei*, *P. rudis*, *P. strigellus*, *P. velutinus*, and one outgroup clade. Phylogenetic analyses showed considerably high support for the *P. conchatus* strain KUMCC18-0047 with MLBS = 92%/ MPBS=90%/ PP = 0.98.



**Fig. 1** – RAxML tree based on a combined sequence dataset of ITS and LSU. Bootstrap values for ML and MP are equal to or greater than 70%, and PP equal or greater than 0.90 are defined as ML/MP/PP. The tree is rooted with *Polyporus melanopus* H6003449 and *P. conifericola* Cui9950. Newly recorded species are indicated in black bold.

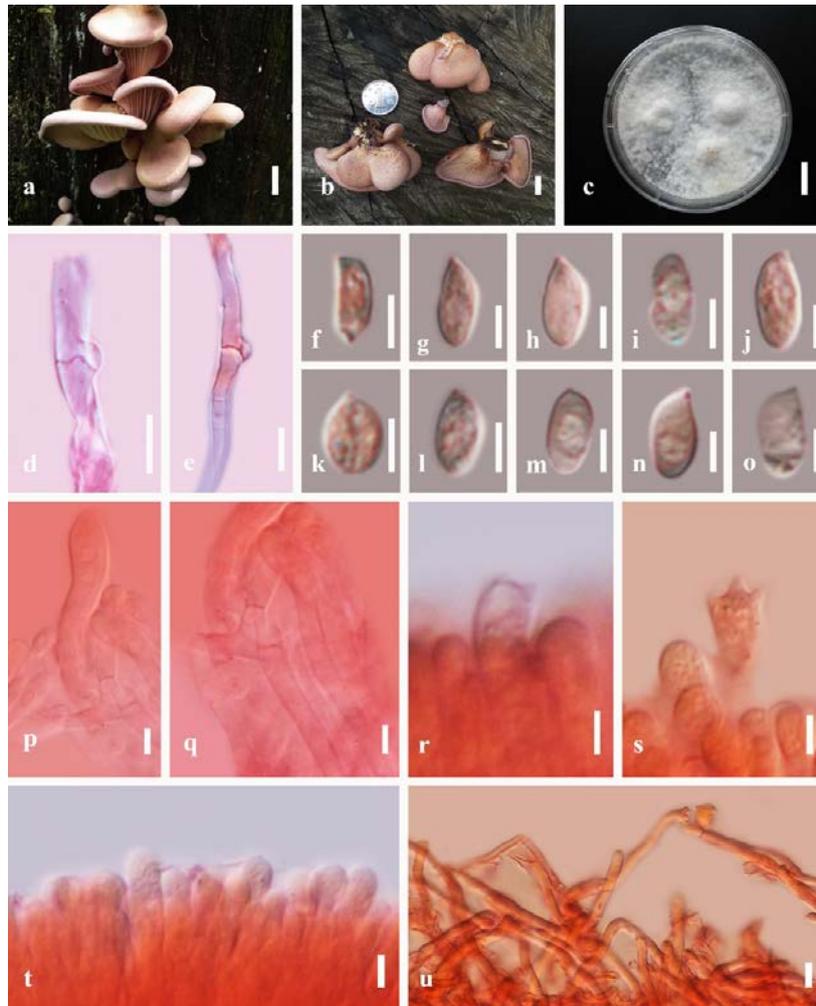
## Taxonomy analyses

*Panus conchatus* (Bull.) Fr., Epicr. syst. mycol. (Upsaliae): 396. 1838.

Fig. 2

Facesoffungi number: FoF 05597

*Basidiocarps* concave, relatively small to medium-sized. *Pileus shape* 1.5–4.5 cm in diameter, initially lateral and reduced, some lateral when mature, usually single when pinhead, slightly to lateral with substantial, overlap expand from base when immature to mature, depressed at center, moderate to deeply indent in side-view when mature, smooth and slippery when wet, soft from center toward margin, sticky at base when fresh, and brittle when dried. *Pileus surface* color variable, initially pinkish (13A2) when young, pale pinkish (13A2), with fading to greyish red (11D4), purple grey (13B2), greyish magenta (13C4), slightly greyish red (11C6), darker toward margin, light orange (5A4) at center, and reddish brown (9E7) when dried, rose (12A4) to bluish red (12A6) on bruising. *Pileus texture* pubescent with netted when young, and usually thin with silky. *Pileus margin* incurved, purple grey (13B2). *Lamellae* sub-distant, deeply decurrentes lines, become forking and anastomosing (joining crossways) at base, usually orange white (6A2), light orange (6A4), yellowish red (8B7), brownish red (8C8) at center toward the base when mature to old, pinkish (13A2), purple grey (13B2–13D2), reddish grey (12B2) toward pileus margin, and greyish ruby (12C3–12E4) when dried. *Stipe* sessile, 0.5–1.7 mm, scaly, with white (13A1) cottony, tough, downy, and strongly attached to the substrate.



**Fig. 2** – Morphological characteristics of *Panus conchatus* strain KUMCC 18-0047. a–b basidiocarps. c the fungal culture incubated at 25°C for 18 days. d–e clamp connection. f–o basidiospores. p–q cheilocystidia. r–s basidia. t metuloids and subhymenium. u generative hyphae. Scale bar: a–c = 1 cm, d–e, p–t = 5 µm, f–o = 2 µm, u = 10 µm.

Hyphal system: *hymenophore* – white (13A1) to yellowish white (4A2), soft and cottony. *Context* dense context layer, up to 2.7–4.3  $\mu\text{m}$  diameter, white to purple grey (13B2) close to the base, mostly generative hyphae, thin-walled with clamp connections, colorless, simple septa, much branched, with a few branches in the distal end, and thick near the base, and lack of skeletal hyphae. *Pileipellis structure* cutis, trichoderm to intricate trichoderm. *Basidia* narrow clavate, with 4 sterigmata, 3.6–4.2  $\times$  22.5–32.3  $\mu\text{m}$  ( $\bar{x}$  = 30). *Basidiospores* mostly oblong to sub-cylindrical, (1.6–)2.4–3.0–3.4(–3.6)  $\times$  (3.6–)5.2–6.2–7.4(–7.5)  $\mu\text{m}$  ( $\bar{x}$  = 2.7  $\times$  5.9  $\mu\text{m}$ ,  $n$  = 50), ellipsoid (1.9–)2.8–3.2–4.0(–4.2)  $\times$  (3.8–)5.2–6.3–7.5(–7.6)  $\mu\text{m}$  ( $\bar{x}$  = 3.3  $\times$  6.2  $\mu\text{m}$ ,  $n$  = 50), and subglobose (2.2–)2.7–3.1–3.4(–4.0)  $\times$  (2.7–)2.9–3.5–4.7(–5.4)  $\mu\text{m}$  ( $\bar{x}$  = 3.0  $\times$  3.5  $\mu\text{m}$ ,  $n$  = 50), white (11A1), overlaid by a hyaline, with thin-walled when maturity, usually occurred suprahilar depression with a distinct hilar appendage. *Lamellae* 1.8–3.5 mm in width, generative hyphae, hyaline with clamp connections are prominent, 2.7–4.2  $\mu\text{m}$  in width ( $\bar{x}$  = 30); skeletal hyphae, hyaline, thin walled, metuloids abundant to occasional on edges of the lamellae, and broad rounded apex of metuloids.

Culture characteristics – initially produced thin white (13A1) mycelium and covered the media surface after incubation for 10 days, and tightly, scattered cottony after incubated 18 days.

Known distribution – widespread in temperate regions.

Additional material examined – CHINA, Yunnan Province, Kunming Botanical Garden, Kunming Institute of Botany, solitary on logwood of *Pinus kesiya* (Royle.) species, 25°07'58"N, 102°44'39"E, on December 2017, T. Luangharn, herbarium number HKAS 97487, culture number KUMCC 18-0047

## Discussion

*Panus conchatus* has previously been described in Yunnan Province, China though the descriptions were based only on morphology and not phylogeny. Few studies on the biologically active compounds of *P. conchatus* have been conducted (Ding et al. 2018). In this study, *P. conchatus* collected from the logwood of *Pinus kesiya* in the temperate climate of Kunming is described based on both morphology and phylogeny. Our collection is in accordance with Pegler (1983), which mentions that *P. conchatus* is often overlapping in a wide range of temperate regions. *P. conchatus* is also widely distributed on hardwoods in subtropical to tropical regions, especially in all types of tropical forests (Corner 1981), including in America and Cameroon (Douanla-Meli 2007). The *Panus* species is also associated with *Betula* spp. and *Populus* spp. in Russia (Zmitrovich et al. 2018).

Our specimen of *P. conchatus* was smaller than the type material described by Corner (1981), which exhibited smaller lamellae with deeply decurrent lines. *Panus conchatus* basidiocarp development are usually occurs as an ephemeral partial veil, and obliterates during basidiocarp expansion (Zmitrovich et al. 2018). We then compared our *P. conchatus* sample with similar species and found that our specimen has some unique differences. The details of the macro-and micro-characteristics are compiled in Table 2. In addition, our results strongly support Pegler (1983), who mentions that this fungus could be distributed in temperate regions.

Few studies have evaluated the phylogeny of *Panus* species without illustrated taxonomy (Moreno et al. 2011, Sotome et al. 2011, Miettinen et al. 2012, Hussein et al. 2014, Zmitrovich & Kovalenko 2016). This study lacks a number of *Panus* collections from China to compare with our collection. Our phylogeny analyses were carried out with combined genes of nLSU and ITS rDNA, while our sequences of TEF1 (MK341057) and RPB2 (MK341058) were added to GenBank (however, they were not included in our analysis, since RPB2 and TEF1 sequences of *Panus* are not available in GenBank). The results revealed that our collection groups of *P. conchatus* collected from China and Tanzania (Hussein et al. 2014, Zmitrovich & Kovalenko 2016) had high supports (ML=92%, MP=90%, PP=0.99%). The clade of *P. fulvus*, *P. strigellus*, and *P. velutinus* serve as a sister group to the *P. conchatus* clade. These results are similar to Hussein et al. (2014), who showed that *P. conchatus* was grouped in the *P. strigellus* clade; however, this fungus has also been reported to form a monophyletic clade with *Lentinus* and *Polyporus* (Hussein et al. 2014). The results of this study will be helpful for the database of Yunnan *Panus*. In this study, we identified

*P. conchatus* from Yunnan Province, China based on morphological characteristics together with phylogenetic data.

**Table 2** The comparison of macro- and micro- characteristics of *Panus conchatus* specimen collected in this study with the type species.

<b>Macro-characteristics</b>	<b><i>P. conchatus</i> (Corner 1981)</b>	<b><i>P. conchatus</i> KUMCC18-0047</b>
Basidiocarps	2–8 cm in width	1–5 cm in width
Basidiocarp shape	concave or plano-infundibuliform	concave
Basidiocarp color	pale lilac-magenta	greyish red (11D5), purple grey (13B2) to greyish magenta (13C4)
Basidiocarp surface	smooth when wet	silky and soft when young to mature
Margin	entire or slightly incurved	incurved
Stipe	short (~6 cm) with irregular	sessile or short
Lamellae size	3.0–3.5 mm in width, and 4–5 ranks	1.8–3.5 mm in width
Lamellae color	dingy cream to pale ochraceous, pinkish near the entire edge	reddish grey (12B2), orange white (6A2), brownish red (8C8), and pinkish (13A2) to purple grey (13B2–13D2) when mature
Lamellae shape and surface	deeply decurrent, abundant of metuloids, rather crowded, white thin, soft and coriaceous when fresh	deeply decurrent lines with soft surface
<b>Micro-characteristics</b>		
Hyphal system	No report	generative hyphae thin walled, with 2.70–4.20 µm in width, with clamp connections, hyaline, and somewhat abundant thick-walled metuloids
Basidiospore shape and size	the shape is not mention, 3.0–3.5 × 6.0–7.0 µm	oblong to sub-cylindrical (2.65 × 5.94 µm), ellipsoid (3.30 × 6.19 µm), and subglobose (3.03 × 3.49 µm)
Basidia shape	no report	narrow clavate
Basidia size	5.0–6.0 × 20.0–28.0 µm, with 4 sterigmata	3.61–4.21 × 22.45–32.25 µm ( $\bar{x}$ = 30), with 4 sterigmata

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