A new species of *Cantharellus* (Cantharellales, Basidiomycota, Fungi) from subalpine forest in Yunnan, China

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**Abstract**

*Cantharellus versicolor* is described and illustrated as a new species based on morphological and molecular characters. The most significant features to distinguish the new species from other known *Cantharellus* are its extremely fleshy, turning gray after injury and with black floccose-fibrillose scales composed of thick-walled and irregular, erect hyphae on the pileus. It is described from the subalpine belt of Shangri-La, northwestern Yunnan, China. Phylogenetic analysis of the transcription elongation factor 1-alpha sequence data further support its systematic position in the subgenus *Cantharellus* and its description as a new species.

**Keywords:** biodiversity, phylogeny, subalpine distribution, *tef1* gene

**Introduction**

Recent surveys of the genus *Cantharellus* Fr. (1821) throughout the world (Buyck, 2012, 2014; Buyck *et al.* 2012, 2013, 2014; Buyck & Randrianjohany, 2013; Foltz *et al.* 2013) have originated a new interest in the genus in China and also some papers on Chinese chanterelloid fungi have recently been published (Shao *et al.* 2011, 2012, 2014; Tian *et al.* 2012). Chanterelles widely distribute in China from northeastern Jilin to central region Henan to southwestern Yunnan Province. In Yunnan, chanterellloid fungi have the slang called “jiyoujun”referring to the frequently light yellow color of many species. As one of the most popular edible mushrooms in market during the harvesting season from May to September, chanterelles are mainly collected from subtropical even tropical regions, seldom from subalpine area. Actually, diversity of the chanterelles exists in the underexplored subalpine region by recent reports (Kumari *et al.* 2012; Tian *et al.* 2012; Vishwakarma and Bhatt, 2013; Das *et al.* 2015).Ongoing taxonomic investigations on chanterelles in Yunnan, China, revealed a new chanterelle distributed in the subalpine mixed forest of Shangri-La (northwestern Yunnan, China). It is here described as *Cantharellus versicolor*, which has specific macromorphological characteristics by extremely fleshy, staining gray after injury and with black floccose-fibrillose scales on the pileus.

As the most efficient marker to characterize closely related species in the genus *Cantharellus* (Buyck *et al.* 2011), transcription elongation factor 1-alpha (*tef1*), a protein coding gene, was analyzed for phylogeny. The systematic placement and taxonomic status are supported by sequence data from the *tef1* gene and this contribution adds another new species to the highly diverse mycota of *Cantharellus* in China.

**Material and methods**

**Morphological study**

Macroscopic descriptions are based on fresh materials and color notations follow the color codes of Kornerup and
Wanscher (1961). Microscopic characters were examined from dried basidiomata using a Nikon E400 microscope (10 × 100)(Nikon, Tokyo) from tissue sections rehydrated in 5% and 10% KOH solution before observation. Line drawings were made with the aid of a drawing tube (Y-IDT, Nikon, Tokyo). At least 20 basidiospores and 10 basidia were measured from each specimen; (m/n/p) indicates measurements based on m basidiospores from n basidiomata in p collections. Basidiospores dimensions are given following the form (a−b−c−d), with b−c containing at least 90% of all values and the extremes (a, d) between parentheses. Q indicates the basidiospores length/width ratio, with Qm denoting the average Q of all basidiospores ± sample standard deviation. The holotype and additional examined materials have been deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

Molecular study

DNA extraction, PCR and sequencing

DNA was extracted according to conventional CTAB method from fresh material or from dried basidiomata stored in silica gel. The primers, Tef1R and Tef1RF (Morehouse et al. 2003), were used to amplify part of the transcription elongation factor 1-alpha (tef1) gene using a Biometra gradient thermal cycler (070-801, Göttingen). PCR products were purified using Sangon’s purification kit. Sequencing was accomplished by Sangon company (Shanghai, China) on an ABI 3730XL automatic sequencer by using the same primer pairs. A maximum likelihood tree based on pairwise alignment of sequences generated from these samples was constructed using MEGA 6.06 (Tamura et al. 2011). New sequences were submitted to the GenBank and accession numbers are listed in the Table 1.

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Boldface: data generated in this study.
Phylogenetic analysis
Sequences of the tef1 gene were sampled from previous studies (Buyck et al. 2011; Buyck & Hofstetter, 2011; Buyck et al. 2013; Foltz et al. 2013; Buyck et al. 2014) and complemented with newly generated sequences (Table 1). Two outgroup sequences from the genus Craterellus and Hydnum, and 48 sequences representative of the genus Cantharellus were used for phylogenetic analyses. Alignment of nucleotide sequences was performed by ClustalX version 1.81 (Thompson et al. 1997). A maximum likelihood (ML) analysis was analyzed by MEGA 6.06 on nearly complete sequence of tef1, using a GTR model with a gamma distribution. The tree with the highest log likelihood is shown. 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 by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach.

Results

Cantharellus versicolor S. C. Shao & P. G. Liu, sp. nov. Fig. 1.
MycoBank no.: MB 810623;

![Image](image_url)

Etymology: the species epithet refers to the darkening characteristic of upon injury.

Holotype: CHINA. Yunnan Prov.: elev. 3294 m, Diqing Tibetan Autonomous Prefecture, Shangri-La county, 23 July 2007., X.F. Tian 161 (HKAS 55762).

Basidiomata extremely fleshy and compact, medium-sized, 60–100 mm in height. Pileus small, 20–30 mm in diam., sandy brown (5C4–5C5) to dark brown (5B8–5C8), firstly applanate with involute margin, becoming more or less plane with age; surface entirely covered with dark brown (5F4) scales, often developing gray after handling. Context solid, fleshy, off-white to pale yellow (1A2), 5–7 mm thick at mid-radius, turning dark brown (5E8) when handled or upon injury.

Odor mild, fungic. Subhymenium shortly decurrent, composed of relatively few and shallow veins, rarely forking and anastomosing, yellow to orange yellow (3A4–4A5). Stipe solid, subcylindrical, 60–100 × 8–10 mm, concolorous or slightly paler than the subhymenium, turning gray after injury. Basidiospores print not obtained.

FIGURE 2. The tree obtained from the Maximum likelihood analysis of tef1 gene for a 50 sequences dataset. Branches are supported based on a 1000 replicates of bootstrap and associated support values are indicated above branches. The placement of Cantharellus versicolor is indicated by the gray box. The right in boldface numerals shows subgenus, clade 1: subgenus Cantharellus; clade 2: subgenus Rubrinus; clade 3: subgenus Cinnabarinus; clade 4: subgenus Parvocantharellus and the outgroup Craterellus tubaeformis, Hydnum repandum.

Basidiospores oval to ellipsoid, smooth, colorless and hyaline, thin-walled, (8.5–) 9.0–10.0 × 5.0–6.0 μm, Q = (1.47–) 1.50–1.78, Qm = 1.65±0.1. Basidia narrowly clavulate to subcylindrical, mostly 83–103 × 7–12 μm, with (2–) 4 (–5) sterigmata. Cystidia absent. Pileipellis composed of two types of hyphae, with irregular, erected, thick-walled hyphae in the scales and thin-walled interwoven hyphae away from the squamules; both composed of similar,
subcylindrical hyphae, measuring 5.5–14.0 μm diam., with pale yellow tint. Subhymenium trama composed of filamentous hyphae 3.0–8.0 μm diam., colorless, thin-walled; clamp connections in all tissues.

Habit, habitat and distribution: caespitose, under trees of Abies fabri (Mast.) Craib. (Pinaceae) forest.


Phylogenetic analysis
The full sequence alignment included 855 characters. The analyzed data set included 393 variable sites in which 330 characters were parsimony-informative. Data partitioning that maximizes likelihood was the one for three partitions (tef1 1st, 2nd and 3rd). The most likely tree (ln = -3128.8952) based on 1000 searches is depicted in Fig. 2 with associated bootstrap values. Phylogenetic analyses show (Fig. 2) that C. versicolor belongs in clade 1 (MLBS=95 %) which corresponds to the subgenus Cantharellus (Corner, 1966: 29), defined by the presence of the type species, C. cibarius (Fries 1821: 318).

Discussion
The new species is morphologically similar to C. ianthinus and C. lilacinus (Corner 1966: 47, 49) with brownish squamules on the cap and similar size of basidiospores, but it differs from those species by its extremely fleshy basidiomata, lacking the purplish tint, much longer basidia, somewhat wider basidiospores and the characteristic of staining gray when handled or injured.

In addition to these morphological features, under the microscope, the black floccose-fibrillose pileipellis characterized by the composition of thick-walled and irregular erect hyphae, ovate-oblong basidiospores up to 10 μm long and narrowly clavulate basidia up to 100 μm in length. The thick-walled hyphae were considered as one of the main characters allowing for the morphological subdivision of the genus and for the definition of the subgenus Cantharellus (Eyssartier & Buyck, 2001a, 2001b), which was also supported in a tef1 gene phylogeny (Buyck & Hofstetter, 2011).

When discussing the results of our phylogenetic analysis, a few limitations of our data should be mentioned. Although the tef1 gene is considered to be the most effective molecular marker for the delimitation of species in genus Cantharellus (Buyck et al. 2011; Buyck & Hofstetter, 2011; Buyck et al. 2013; Foltz et al. 2013) even within the C. cibarius complex, highly similar species matching C. versicolor in GenBank using BLAST are not present. The tef1 sequence of our species showed rather low similarity to other sequences deposited in GenBank for Cantharellus. Yet, our data fully supported its suggested placement in the subgenus Cantharellus. All of the subgenus Cantharellus constituent species are northern hemisphere taxa that have abundant clamps. With the exception of the North American C. quercophilus (Buyck et al. 2010: 19) and, to a lesser degree, also the American C. tenuithrix (Buyck & V. Hofstetter 2011: 43), all of the sequenced species in this clade possess distinctly thick-walled hyphal extremities in the pileipellis.

Most of the species are yellow, but this color is sometimes mixed with greenish, brownish, vinaceous to lilac-purple pigments. Some species have a squamulose cap, although the squamulae are less developed or more appressed compared to the majority of species that compose clade 2. The new species showed closer relationship and formed a subclade in clade 1 with C. phasmatis (Foltz et al. 2013: 454), C. flavus (Foltzet et al. 2013: 456), C. tenuithrix, C. cibarius, C. quercophilus and C. altipes (Buyck et al. 2011: 39).

Acknowledgments
The first author thanks Dr. Fu-Qiang Yu (Kunming Institute of Botany, CAS) for offering some samples. This work was partially supported by the National Natural Science Foundation of China (No. 31400021 & 31270075). The Joint Founds of the National Science Foundation of China and Yunnan Province Government (U1202262) and Key Laboratory for Plant Diversity and Biogeography of Eastern Asia, Kunming Institute of Botany, Chinese Academy of Sciences (No.0806361121). Acknowledgments by B. Buyck are also due for financial support for sequencing through the ATM 2014 “Emergences” (Dirs. P. Janvier & S. Peigné) from the National Museum of Natural History, Paris.
References


