



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, cantharelloid 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 Q_m 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.

TABLE 1. Voucher table indicating the personal field identification (Taxon), collector and collection number (voucher), herbarium accession number (HKAS, PC barcode number and Field Museum of Natural History (F)), country of origin and GenBank accession numbers of *tef1* gene. Abbreviations: BB: Bart Buyck; GE: Guillaume Eyssartier; Tian: Xiao-Fei Tian; Yu: Fu-Qiang Yu; AFTOL: Assembling the Tree Of Life consortium. C.: *Cantharellus*.

Taxon	Voucher extraction No./ coll. No.	Herb. Acc. No.	Origin	<i>tef1</i>
<i>Cantharellus addaiensis</i> Henn.	495/BB 98.033 neotype	PC0084717	Tanzania	JX192992
<i>C. addaiensis</i>	267/BB 98.057	PC0084718	Tanzania	JX192976
<i>C. altipes</i>	BB 07.115	PC0084082	USA	GQ914943
<i>C. altipes</i>	344/BB 07.162 paratype	PC0084090	USA	GQ914945
<i>C. altipes</i>	318/BB 07.019 holotype	PC0084085	USA	GQ914939
<i>C. amethysteus</i> (Quel.) Sacc.	352/BB 07.309	PC0084071	Slovakia	GQ914954
<i>C. amethysteus</i>	349/BB 07.284	PC0084070	Slovakia	GQ914953
<i>C. appalachiensis</i> R.H.Petersen	BB 07.123	PC0084075	USA	GQ914979
<i>C. cibarius</i>	AFTOL-ID 607	PC0084078	AFTOL-ID 607	DQ059050
<i>C. cibarius</i>	479/GE 07.025	PC0084088	France	GQ914949
<i>C. cibarius</i>	351/BB 07.300	PC0084077	Slovakia	GQ914950
<i>C. cinnabarinus</i> (Schwein.) Schwein.	BB 04.263	PC 0084092	USA	GQ914983
<i>C. cinnabarinus</i>	326/BB 07.053	PC0084093	USA	GQ914984
<i>C. cinnabarinus</i>	312/BB 07.001 neotype	PC0084094	USA	GQ914985
<i>C. decolorans</i> Eyssart. & Buyck	469/BB 08.278 epitype	PC0084098	Madagascar	GQ914968
<i>C. aff. decolorans</i>	57/BB 06.146	PC0084757	Madagascar	JX192964
<i>C. aff. decolorans</i>	466/BB 08.243	PC0084733	Madagascar	JX192987
<i>C. aff. decolorans</i>	20/BB 06.168	PC0124633	Madagascar	JX192999

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TABLE 1. (Continued)

Taxon	Voucher extraction No./ coll. No.	Herb. Acc. No.	Origin	<i>tef1</i>
<i>C. ferruginascens</i> P.D. Orton	348/BB 07.283	PC0084099	Slovakia	GQ914952
<i>C. isabellinus</i> var. <i>parvisporus</i> Eyssart. & Buyck	256/BB 98.020 holotype	PC0084753	Tanzania	JX192972
<i>C. isabellinus</i> var. <i>parvisporus</i>	249/BB 98.037 paratype	PC0084100	Tanzania	GQ914966
<i>C. lateritius</i> (Berk.) Singer	BB 07.004	PC0084101	USA	GQ914955
<i>C. lateritius</i>	BB 07.025	PC0084103	USA	GQ914957
<i>C. lateritius</i>	BB 07.058	PC0084105	USA	GQ914959
<i>C. lewisii</i> Buyck & V. Hofstetter	BB 06.320	PC0084072	USA	GQ914960
<i>C. lewisii</i>	314/BB 07.003 holotype	PC0084074	USA	GQ914962
<i>C. lewisii</i>	301/BB 02.197 paratype	PC0084073	USA	GQ914961
<i>C. lilacinopruinatus</i> Hermitte, Eyssart. & Poumarat	347/BB 07.221	PC0084106	Slovakia	GQ914951
<i>C. phasmatis</i>	C057	C0171587F	USA	JX030417
<i>C. phasmatis</i>	C074	C0171588F	USA	JX030418
<i>C. quercophilus</i>	636/BB 07.097 holotype	PC0084726	USA	JX192981
<i>C. subincarnatus</i> subsp. <i>rubrosalmoneus</i> Buyck & V. Hofstetter	13/BB 06.080 holotype	PC0084727	Madagascar	JX192962
<i>C. subincarnatus</i> subsp. <i>rubrosalmoneus</i>	55/BB 06.096 paratype	PC0084755	Madagascar	JX192963
<i>C. tabernensis</i> Feib. & Cibula	BB 07.056	PC0084115	USA	GQ914974
<i>C. tabernensis</i>	BB 07.119	PC0084116	USA	GQ914976
<i>C. tabernensis</i>	BB 07.118	PC0084118	USA	GQ914978
<i>C. tanzanicus</i> Buyck & V. Hofstetter	268/BB 98.040 holotype	PC0084728	Tanzania	JX192977
<i>C. aff. tanzanicus</i>	59/BB 06.148	PC0084127	Madagascar	JX192965
<i>C. aff. tanzanicus</i>	60/BB 06.149	PC0084128	Madagascar	JX192966
<i>C. tenuithrix</i> Buyck & V. Hofstetter	343/BB 07.125 holotype	PC0084084	USA	GQ914947
<i>C. tenuithrix</i>	322/BB 07.035 paratype	PC0084087	USA	GQ914946
<i>C. texensis</i> Buyck & V. Hofstetter	BB 07.113/	PC0084095	USA	GQ914986
<i>C. texensis</i>	341/BB 07.120 paratype	PC0084096	USA	GQ914987
<i>C. texensis</i>	317/BB 07.018 holotype	PC0084097	USA	GQ914988
<i>C. tomentosus</i> Eyssart. & Buyck	500/BB 98.060 holotype	PC0084732	Tanzania	JX192995
<i>C. tomentosus</i>	248/BB 98.038 paratype	PC0084121	Tanzania	GQ914965
<i>C. versicolor</i>	Tian 160	HKAS 55761	China	KM893857
<i>C. versicolor</i>	Yu 24	HKAS 58242	China	KM893856
<i>Craterellus tubaeformis</i> (Fr.) Quél.	BB 07.293	PC0084122	Slovakia	GQ914989
<i>Hydnum repandum</i> L.	356/BB 07.341/MTS3757	PC0084749	Slovakia	JX192980

Boldface: data generated in this study.

Phylogenetic analysis

Sequences of the *tefl* 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 *tefl*, 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;

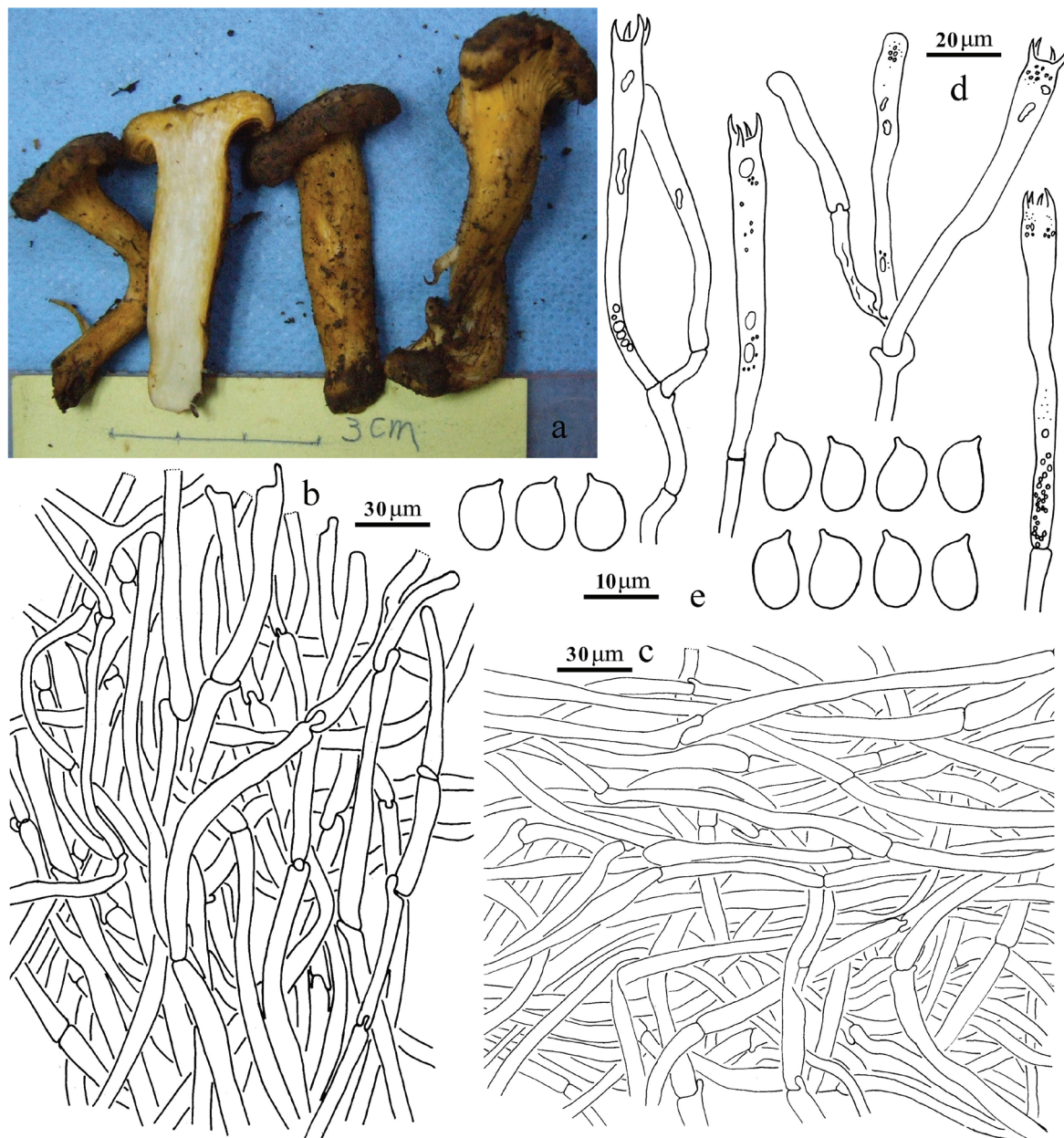


FIGURE 1. *Cantharellus versicolor* (HKAS 55762, holotype). a. Basidiocarps, b. Structure of squamules on pileus, c. Pileipellis from an area not covered by squamules, d. Basidia, e. Basidiospores.

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.

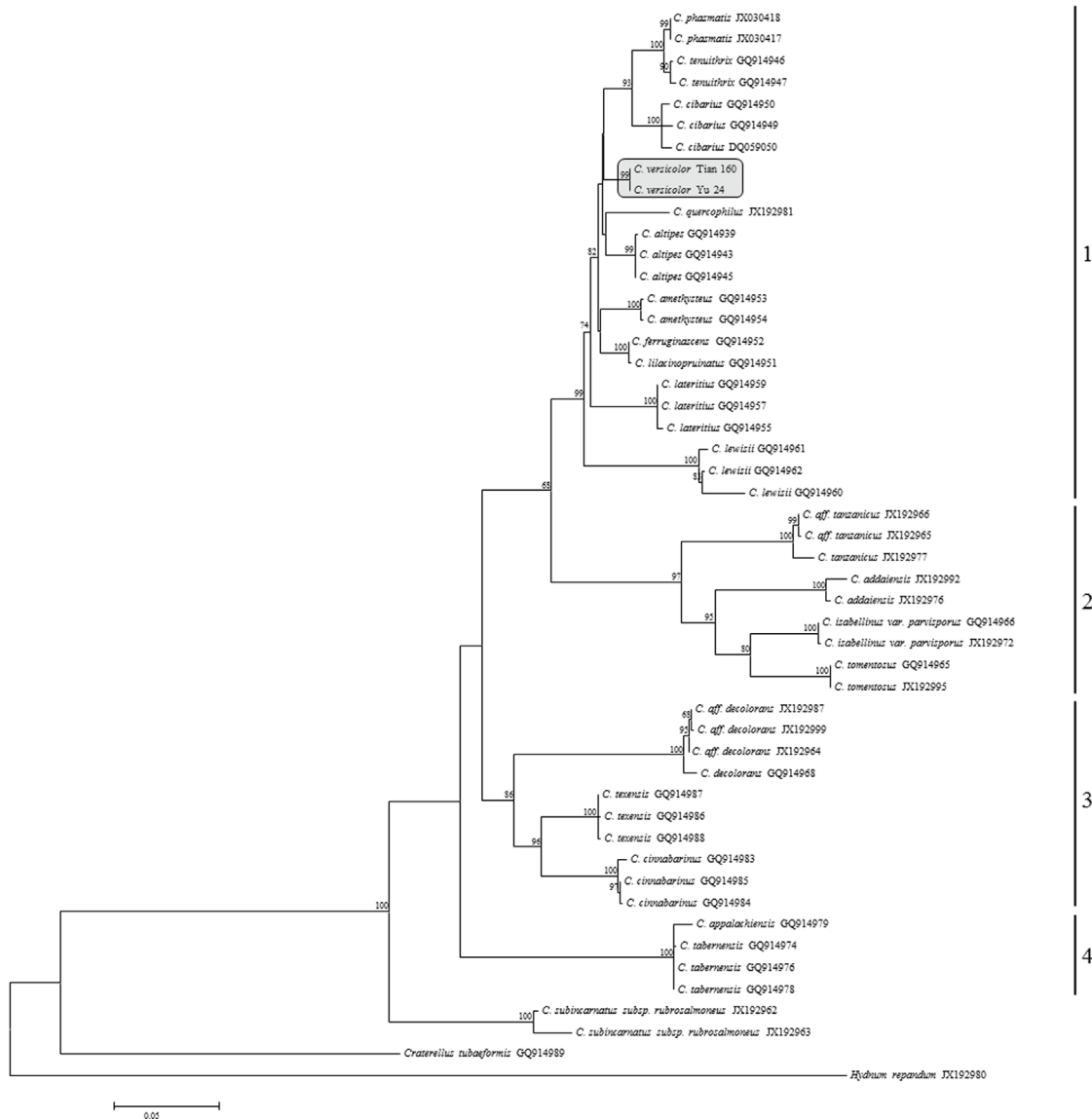


FIGURE 2. The tree obtained from the Maximum likelihood analysis of *tef1* gene for a 50 sequences dataset. Branches are supported based on a 1000 replicates of bootstrap and associated support values are indicated above branches. The placement of *Cantharellus versicolor* is indicated by the gray box. 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, Q_m = 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.

Specimens examined: CHINA. Yunnan Prov.: elev. 3294 m, Diqing Tibetan Autonomous Prefecture, Shangri-La county, 22 July 2007, *X.F. Tian 154* (HKAS55757), *X.F. Tian 155* (HKAS 55758); *ibid.*, 23 July 2007, *X.F. Tian 160* (HKAS 55761), *X.F. Tian 162* (HKAS 55763), *X.F. Tian 163* (HKAS 55764); Deqin mushroom market, 2 December 2008, *F.Q. Yu 24* (HKAS 58242).

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 (*tefl* 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 *tefl* gene phylogeny (Buyck & Hofstetter, 2011).

When discussing the results of our phylogenetic analysis, a few limitations of our data should be mentioned. Although the *tefl* 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 *tefl* 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* (Foltz *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 Funds 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” (Drs. P. Janvier & S. Peigné) from the National Museum of Natural History, Paris.

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