

# *Afrocantharellus* gen. stat. nov. is part of a rich diversity of African *Cantharellaceae*

Donatha D. Tibuswa<sup>1,2\*</sup>, Sanja Savić<sup>2</sup>, Leif Tibell<sup>2</sup>, and Amelia K. Kivaisi<sup>1</sup>

<sup>1</sup>Department of Molecular Biology and Biotechnology, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania (Permanent address); corresponding author e-mail: dtibuswa@yahoo.co.uk

<sup>2</sup>Department of Systematic Biology, Institute for Organismal Biology, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

**Abstract:** A new genus in the *Cantharellaceae*, *Afrocantharellus*, is recognized based on results from phylogenetic analyses of rDNA LSU and concatenated LSU/5.8-ITS2/ATP6 data. It was previously recognized as a subgenus, but comprehensive fieldwork and the acquisition of numerous sequences for previously neglected African *Cantharellus* species formed the basis for a reappraisal of generic and species delimitations. *Afrocantharellus* is characterized morphologically by the basidiomes having thick, distantly spaced diverging folds of variegated colour. In contrast to most of *Cantharellus*, *Afrocantharellus* mostly lacks clamp connections. Phylogenies of *Cantharellus* and *Afrocantharellus* based on LSU and a concatenated data set are provided, along with descriptions of and a key to the four species and one form of *Afrocantharellus* recognized. Six new combinations are made.

**Key words:**

Africa  
ATP6  
*Cantharellus*  
ITS  
LSU  
Molecular phylogeny  
Tanzania

**Article info:** Submitted: 25 February 2012; Accepted: 16 May 2012; Published: 21 June 2012.

## INTRODUCTION

*Cantharellaceae* comprise mycorrhizal and saprobic fungi, which in most cases have a vase-shaped or funnel-shaped basidiome and a spore-bearing smooth, wrinkled, veined or folded lower side. *Cantharellus*, as presently delineated, includes about 23 species in North America, seven in South America, seven in Australia, nine in Europe, three in New Zealand, 46 in Africa, and 19 in Asia (Eyssartier 2003, Tibuswa *et al.* 2008, Buyck & Hofstetter 2011, Buyck *et al.* 2011, Eyssartier *et al.* 2009, Shao *et al.* 2011). *Cantharellus* includes several well-known and highly esteemed edible species. In Africa, *Cantharellus* species are widely collected and sold on local markets. A revision of African *Cantharellus* from the Belgian Congo was given by Heinemann (1958), who later (Heinemann 1966) also treated species from Katanga, describing *C. platyphyllus* and *C. symoensii* as new. In a review of edible mushrooms from Burundi (Buyck 1994), a further species, *C. splendens*, was described, and others are mentioned in a list of *Cantharellus* species from the same country (Buyck & Nzigidahera 1995). Further notes on *Cantharellus* from Africa, including detailed investigations of some type specimens, were published by Eyssartier & Buyck (1998). A list of and key to *Cantharellus* species known from Tanzania was provided by Buyck *et al.* (2000). Nomenclatural notes and descriptions of new subgenera and sections in *Cantharellus* were published by Eyssartier & Buyck (2001).

### Molecular studies of the ‘cantharelloid clade’

The phylogeny of the ‘cantharelloid clade’, including

*Cantharellus* and the closely related *Craterellus*, has recently been investigated using molecular data, and reviewed by Moncalvo *et al.* (2006). Incongruence was noted between relationships as reconstructed from different genes, particularly with respect to the placement of *Tulasnella*. *Cantharellus* and *Craterellus* consistently were monophyletic and sister-groups in analyses based on LSU, SSU, mtSSU, and *RPB2* sequences. Large subunit nuclear encoded rDNA (LSU) and or ITS sequences have been used for elucidating the phylogeny of or in *Cantharellales* in several papers (Feibelman *et al.* 1994, Feibelman *et al.* 1997, Hibbett *et al.* 1997, Pine *et al.* 1999, Li *et al.* 1999, Dahlman *et al.* 2000, Hibbett *et al.* 2000, Binder & Hibbett 2002, Moncalvo *et al.* 2006, Olariaga *et al.* 2009). In *Cantharellaceae*, according to Feibelman *et al.* (1994), the ITS region is unusually long and highly variable in length, especially in the chanterelles (see also Dunham *et al.* 2003). Additionally, significant length variability in ITS and morphology of North America *Cantharellus cibarius*-like chanterelles has been demonstrated, suggesting a species complex masked by a common morphology (Feibelman *et al.* 1994, Dunham *et al.* 2003, Pilz *et al.* 2003). Moncalvo *et al.* (2006) recommended the use of protein-coding genes such as *RPB2* for the reconstruction of evolutionary relationships in the cantharelloid clade. This, however, primarily had a background in incongruent placement of *Tulasnella* with different datasets, whereas LSU still seems to efficiently resolve relationships, also in *Botryobasidium* and *Tulasnella*. Problems in using LSU datasets include long-branch attraction in some types of analyses, particularly in distance and parsimony-based analyses (Moncalvo *et al.*

© 2012 International Mycological Association

You are free to share - to copy, distribute and transmit the work, under the following conditions:

**Attribution:** You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).

**Non-commercial:** You may not use this work for commercial purposes.

**No derivative works:** You may not alter, transform, or build upon this work.

For any reuse or distribution, you must make clear to others the license terms of this work, which can be found at <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>. Any of the above conditions can be waived if you get permission from the copyright holder. Nothing in this license impairs or restricts the author's moral rights.

2006). Alignment problems are also sometimes encountered. These, however, are much more pronounced at the order or family level, but are manageable and cause much less data loss within the genera (Moncalvo *et al.* 2006).

Although LSU- and mtSSU-based analyses previously have been shown to efficiently resolve phylogenetic relationships in *Cantharellaceae* (Moncalvo *et al.* 2006), here data from additional regions was utilized. ATP6 (which codes for ATP-ase subunit 6) has so far not been used for phylogenetic inference in *Cantharellaceae*, but Kretzer & Bruns (1999) successfully resolved phylogenetic relationships in *Boletales* using this protein-coding gene. Recently, a maximum likelihood analysis was employed on a dataset for the protein coding gene *tef-1*, leading to the recognition of a new North American *Cantharellus* species (Buyck *et al.* 2011) and including discussions of species delimitation in the *Cantharellus cibarius* complex in the southeastern USA (Buyck & Hofstetter 2011). Buyck & Hofstetter (2008) presented preliminary results of a four gene phylogeny for *Cantharellus*, employing mtSSU, LSU, and two protein-coding loci, *tef-1* and *RPB2*, where *ca.* 45 species from four continents were sampled suggesting the recognition of at least six different clades. However, in conclusion those authors stated that more studies on a larger data set were needed for the recognition of further taxa. Although several molecular studies have investigated relationships of the 'cantharelloid clade' (Hibbett *et al.* 1997, 2000, Pine *et al.* 1999, Hibbett & Donoghue 2001, Binder & Hibbett 2002, Larsson *et al.* 2004, Binder *et al.* 2005, Mathney 2005, Moncalvo *et al.* 2006) and *Cantharellus* (Feibelman *et al.* 1997, Dahlman *et al.* 2000, Dunham *et al.* 2003, Thacker & Henkel 2004, Henkel *et al.* 2005), to our knowledge just a few sequences from African species have been published. Considering the high diversity of the genus in Africa, this might well have hampered our understanding of the phylogeny of *Cantharellus* and the 'cantharelloid clade' as a whole.

Thus, the main criticism that can be levelled against the molecular analyses so far published of phylogenetic relationships of *Cantharellus s. lat.* is that the taxon sampling has been quite limited. The species sampled have been almost exclusively from the Northern Hemisphere, despite the rich diversity of *Cantharellus* in other parts of the world. The diversity of *Cantharellus* in Africa is particularly exceptional, and the inclusion of data on African *Cantharellus* may thus be expected to contribute substantially to alleviate the lack in comprehensiveness and phylogenetic relationships in current analyses.

### Current species recognition in *Cantharellus*

In *Cantharellus*, as currently circumscribed, the distinction between the species still often remains extremely subtle given the few and variable morphological characters available for species recognition (Buyck & Hofstetter 2011). For example the name *C. cibarius* (or '*C. cf. cibarius*') often refers to any yellowish chanterelle, and *C. cibarius* is no doubt the most commonly misapplied name for a chanterelle. When the status of nominal species and morphological variability within the species was not clear, sometimes these 'ambiguous species' were included in species groups or

species complexes. *Cantharellus cibarius*, considered to contain 'several cryptic geographic species' by Moncalvo *et al.* (2006), is the type of *Cantharellus* and this complicates the circumscription of *Cantharellus s. str.* Additionally, Buyck & Hofstetter (2011) stated that many morphologically similar species and infraspecific taxa had been included under *C. cibarius*.

However, with the use of molecular information, there is evidence that a substantial number of unrecognized fungal species are hidden under traditional phenotype-based species names (e.g. Carriconde *et al.* 2008). However, the outcome of recent studies of basidiomycetes based on molecular data varies. In some cases the recognition of morphologically circumscribed species and infrageneric taxa, as monophyletic groups, is not supported (e.g. Geml *et al.* 2006, Frøslev *et al.* 2007, Nagy *et al.* 2012). Thus, species recognition based on molecular data should be adopted when a morphological species concept is inapplicable in the sense that it is not consistent with the genetic information. Not wanting to argue a general, criterion-based 'species concept' (see also Hey 2006), we have for this study searched for congruence between molecular phylogenies and morphological features evaluated *a posteriori* in recognizing taxa.

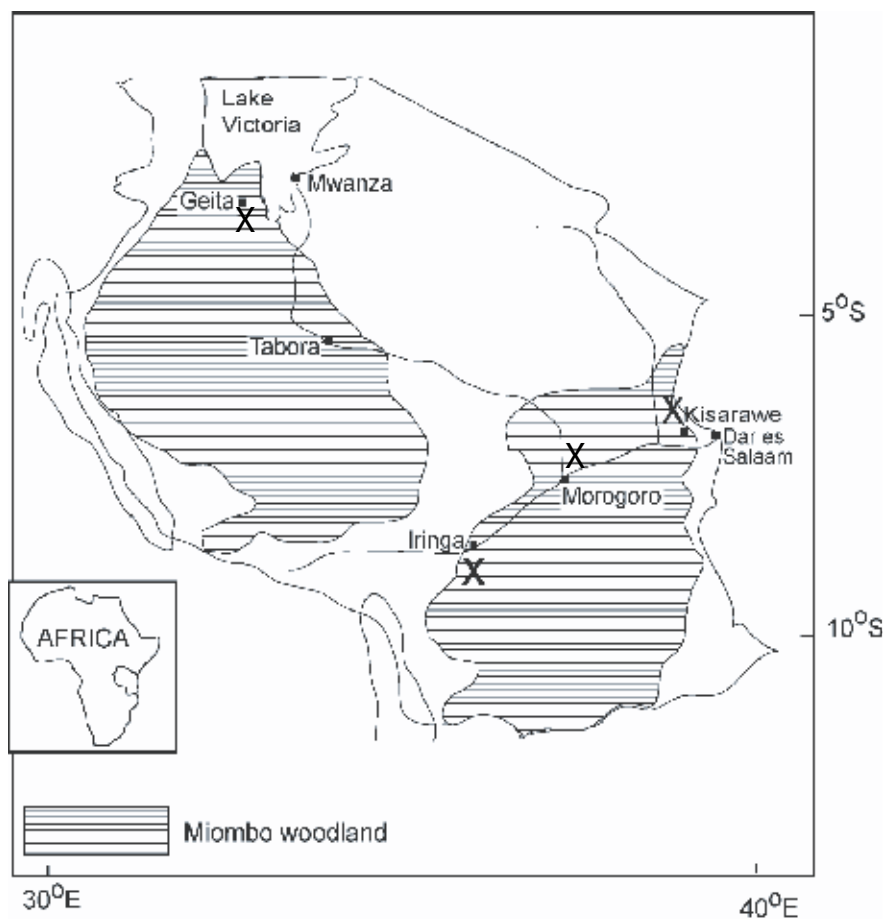
The aim of this study is to contribute to a better understanding and reassessment of the phylogeny of *Cantharellus* based on the inclusion of molecular data derived from the rich diversity of African *Cantharellus* species based on partial LSU, 5.8-ITS2, and ATP6 sequences.

## MATERIALS AND METHODS

### Taxon and sequence sampling

All *Cantharellus* samples were collected by the first author both in the northern and southern parts of Tanzanian miombo woodlands (Fig. 1) in April–June and September–December during four consecutive years (2004–2007). Specimens were preserved either by immediate freezing in saturated brine solution, in CTAB until investigated, or dried overnight at 60 °C for herbarium deposition and further analysis. Microscopic characters were examined as in Tibuhwa *et al.* (2008). This involved recording 40 measurements of each feature from both fresh specimen preserved in CTAB, and dry specimens observed in 10 % ammonium solution in an aqueous solution of Congo red. The estimated size of the measured feature was obtained statistically and presented as: (min) min-SD –  $\overline{AV}$  – max-SD (max) Q, in which min = lowest value recorded for the measured feature, max = highest value,  $\overline{AV}$  = arithmetic mean and SD standard deviation; Q the ratio length/width (Eyssartier *et al.* 2001, Tibuhwa *et al.* 2008). Spore shapes were described according to Bas (1969).

For molecular characterization 5.8S–ITS2 and ATP6 were sequenced for 21 and 20 specimens of *Cantharellus* respectively, and LSU for 36 specimens, including three *Craterellus* species. In total, 77 new sequences were produced. GenBank numbers and voucher specimen information for sequences we generated are listed in Table 1, together with sequences obtained from GenBank. To estimate the phylogenetic position of African *Cantharellus* species as represented by the Tanzanian material, we worked with two



**Fig. 1.** Map showing the distribution of Miombo-woodlands in Tanzania. Approximate positions of collecting sites are marked with 'X'.

datasets: (1) a large LSU dataset; and (2) a more restricted dataset of concatenated LSU/5.8-ITS2/ATP6.

**The first dataset:** The larger dataset LSU comprised 92 taxa of *Cantharellus* and related genera selected for this study. Sequences from GenBank were selected so that if possible at least two sequences representing each species were included. In the selection of representatives of the 'cantharelloid clade' and choice of outgroup we were guided by the results presented by Moncalvo *et al.* (2006). In the large LSU sampling, representatives of *Craterellus*, *Hydnum*, and *Multiclavula* were included representing more remote relatives of *Cantharellus*. *Multiclavula mucida* was used as outgroup.

**The second dataset:** A concatenated data set included LSU/5.8-ITS2/ATP6, forming 28 sets of sequences representing 17 species. We tried to include the same representatives for all three regions; however, the concatenated matrix was not entirely complete, missing three sequences for 5.8-ITS2 and four for ATP6. The ATP6 sampling was limiting this selection. In the ATP6 partition, however, no *Craterellus* sequence was available, and of Northern Hemisphere *Cantharellus* species only two, *viz.* *C. cibarius*, and *C. cinnabarinus* were included. Considering that *C. cibarius* is a frequently misapplied name, it is problematic to combine different sequences available from GenBank under this name. Thus we decided not to include it in our second data set. Moreover, we failed to obtain additional ATP6 sequences from twelve Northern Hemisphere *Cantharellus* species and two *Craterellus* species because of amplification problems and the potential occurrence of paralogs. Interestingly, the

same issue did not arise during the amplification of ATP6 from African species. In addition, we used an amalgamated set for *Clavulina* sequences, combining from GenBank for LSU and 5.8-ITS2 from *Cl. cinerea* with *Clavulina* sp. for ATP6; *Dacrymyces chrysospermus* served as outgroup.

The alignments, together with the trees from the Bayesian analyses (Figs 2–3), have been deposited in TreeBASE (<http://purl.org/phylo/treebase/phylovs/study/TB2:S12709>).

## Molecular study

### DNA extraction, amplification, and sequencing

Total DNA was extracted from the inner part of the basidiomes, preferentially from the hymenium to avoid contamination, following the protocol of the Plant Genomic DNA extraction Kit (VIOGEN). Diluted ( $10^{-1}$  –  $10^{-3}$ ) or undiluted DNA was used for PCR amplifications. The 5' end of the LSU, and 5.8-ITS2 and ATP6 were amplified. Primers used were: (a) for the 5' part of LSU: LR3 and LR5 (Vilgalys & Hester 1990), and forward primer LROR (<http://www.biology.duke.edu/fungi/mycolab/primers.htm#Large> subunit RNA (25-28S) primer sequences) or LCa1 (primer designed for this study: 5'-GTCCGAGTTGTAGATGAG-3'); (b) for amplification of 5.8S-ITS2 part of ITS region see Table 2; (c) for the ATP6: ATP6-2 and ATP6-3 (Kretzer & Bruns 1999).

For PCR amplification of all three regions (LSU, 5.8-ITS2, and ATP6) we used the AccuPower® PCR PreMix (Bioneer, Daejeon, Korea), adding 3  $\mu$ L diluted or undiluted DNA, 1.5  $\mu$ L of each primer (10  $\mu$ M), and water to a total volume

**Table 1.** Specimens and sequences used in this study, with their respective voucher information. GenBank accession numbers in bold represent sequences published here for the first time; corresponding voucher and collector numbers are provided. Other GenBank ID numbers represent sequences already published.

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
1	<i>Afrocantharellus fistulosus</i>	DDT31	TANZANIA: Kisarawe	Tibuhwa 31.2006	<b>JQ976959</b>	—	—
2	<i>A. fistulosus</i>	DDT43	TANZANIA: Kisarawe	Tibuhwa 43.2007	<b>JQ976965</b>	—	—
3	<i>A. platyphyllus</i> f. <i>cyanescens</i>	DDT63	TANZANIA: Morogoro	Tibuhwa 1063.2007	<b>JQ976970</b>	—	—
4	<i>A. platyphyllus</i> f. <i>platyphyllus</i>	DDT78	TANZANIA: Iringa	Tibuhwa 1078.2007	<b>JQ976978</b>	<b>JQ976947</b>	<b>JQ976926</b>
5	<i>A. platyphyllus</i> f. <i>platyphyllus</i>	DDT03	TANZANIA: Morogoro	Tibuhwa 1003.2004	<b>JQ976950</b>	<b>JQ976929</b>	—
6	<i>A. platyphyllus</i> f. <i>platyphyllus</i>	DDT41	TANZANIA: Kisarawe	Tibuhwa 1041.2006	<b>JQ976964</b>	—	—
7	<i>A. splendens</i>	DDT57	TANZANIA: Morogoro	Tibuhwa 1057.2007	<b>JQ976967</b>	<b>JQ976937</b>	<b>JQ976916</b>
8	<i>A. splendens</i>	DDT17	TANZANIA: Geita	Tibuhwa 1017.2005	<b>JQ976956</b>	<b>JQ976932</b>	<b>JQ976911</b>
9	<i>A. symoensii</i>	DDT36	TANZANIA: Kisarawe	Tibuhwa 1036.2005	<b>JQ976961</b>	<b>JQ976934</b>	<b>JQ976914</b>
10	<i>A. symoensii</i>	DDT04	TANZANIA: Morogoro	Tibuhwa 1004.2005	<b>JQ976951</b>	—	—
11	<i>A. symoensii</i>	DDT66	TANZANIA: Iringa	Tibuhwa 1066.2007	<b>JQ976971</b>	<b>JQ976940</b>	<b>JQ976919</b>
12	<i>A. symoensii</i>	DDT11	TANZANIA: Morogoro	Tibuhwa 1011.2005	<b>JQ976953</b>	—	—
13	<i>A. symoensii</i>	DDT67	TANZANIA: Iringa	Tibuhwa 1067.2007	<b>JQ976972</b>	<b>JQ976941</b>	<b>JQ976920</b>
14	<i>A. symoensii</i>	DDT14	TANZANIA: Geita	Tibuhwa 1014.2004	<b>JQ976955</b>	—	—
15	<i>Botryobasidium isabellinum</i>				AF393047	—	DQ534597.1
16	<i>C. appalachiensis</i>				DQ898690	—	—
17	<i>C. appalachiensis</i>				HM750916	—	—
18	<i>C. cascadiensis</i>				AY041159	—	—
19	<i>C. cascadiensis</i>				AY041158	—	—
20	<i>C. cascadiensis</i>				AY041161	—	—
21	<i>C. cascadiensis</i>				AY041160	—	—
22	<i>C. cibarius</i> var. <i>cibarius</i>				AY041156	—	—
23	<i>C. cibarius</i> var. <i>cibarius</i>				AY041155	—	—
24	<i>C. cibarius</i> var. <i>cibarius</i>				AY041157	—	—
25	<i>C. cibarius</i> var. <i>roseocanus</i>				AY041152	—	—
26	<i>C. cibarius</i> var. <i>roseocanus</i>				AY041153	—	—
27	<i>C. cibarius</i> var. <i>roseocanus</i>				AY041154	—	—
28	<i>C. cibarius</i> var. <i>roseocanus</i>				AY041151	—	—
29	<i>C. cibarius</i> var. <i>multiramis</i>				HM750920	—	—
30	<i>C. cibarius</i>	SS574	SWEDEN: Uppland	Olariaga & Felipe 2005/503752	<b>JQ976981</b>	—	—
31	<i>C. cibarius</i>				EU522825	—	—
32	<i>C. cibarius</i>				AJ406428	—	—
33	<i>C. cibarius</i>				HM750927	—	—
34	<i>C. cibarius</i>				AY745708	—	—
35	<i>C. cibarius</i>				DQ898693	—	—
36	<i>C. cibarius</i> var. <i>longipes</i>				HM750924	—	—
37	<i>C. cinnabarinus</i>				AY041168	—	—
38	<i>C. cinnabarinus</i>				DQ898692	—	—
					—	—	DQ120944
					—	DQ898649	—
39	<i>C. congolensis</i>	DDT77	TANZANIA: Morogoro	Tibuhwa 1077.2007	<b>JQ976977</b>	<b>JQ976946</b>	<b>JQ976925</b>
40	<i>C. congolensis</i>	DDT76	TANZANIA: Iringa	Tibuhwa 1076.2007	<b>JQ976976</b>	<b>JQ976945</b>	<b>JQ976924</b>

Table 1. (Continued).

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
41	<i>C. densifolius</i>	DDT40	TANZANIA: Kisarawe	Tibuhwa 1040.2006	<b>JQ976963</b>	<b>JQ976935</b>	<b>JQ976915</b>
42	<i>C. densifolius</i>	DDT58	TANZANIA: Morogoro	Tibuhwa 1058.2006	<b>JQ976968</b>	<b>JQ976938</b>	<b>JQ976917</b>
43	<i>C. floridulus</i>	DDT33	TANZANIA: Morogoro	Tibuhwa 1033.2006	<b>JQ976960</b>	—	<b>JQ976913</b>
44	<i>C. floridulus</i>	DDT38	TANZANIA: Morogoro	Tibuhwa 1038.2005	<b>JQ976962</b>	—	—
45	<i>C. formosus</i>				AY041166	—	—
46	<i>C. formosus</i>				AY041164	—	—
47	<i>C. formosus</i>				AY041165	—	—
48	<i>C. garnierii</i>				AY392767	—	—
49	<i>C. garnierii</i>				AY392768	—	—
50	<i>C. isabellinus</i>				HM750931	—	—
51	<i>C. isabellinus</i>	DDT30	TANZANIA: Morogoro	Tibuhwa 1030.2006	<b>JQ976958</b>	—	—
52	<i>C. isabellinus</i> var. <i>parvisporus</i>	DDT12	TANZANIA: Morogoro	Tibuhwa 1012.2004	<b>JQ976954</b>	<b>JQ976931</b>	<b>JQ976910</b>
53	<i>C. isabellinus</i> var. <i>parvisporus</i>	DDT22	TANZANIA: Geita	Tibuhwa 1022.2005	<b>JQ976957</b>	<b>JQ976933</b>	<b>JQ976912</b>
54	<i>C. lateritius</i>				DQ898694	—	—
55	<i>C. minor</i>				DQ898691	—	—
56	<i>C. minor</i>				HM750923	—	—
57	<i>C. pallens</i>	SS577	SWEDEN: Uppland	Danell & Olariaga 2005 (503727)	<b>JQ976984</b>	—	—
58	<i>C. persicinus</i>				AY041169	—	—
59	<i>C. pseudocibarius</i>	DDT02	TANZANIA: Morogoro	Tibuhwa 1002.2004	<b>JQ976949</b>	<b>JQ976928</b>	<b>JQ976908</b>
60	<i>C. pseudocibarius</i>	DDT05	TANZANIA: Geita	Tibuhwa 1005.2004	<b>JQ976952</b>	<b>JQ976929</b>	<b>JQ976909</b>
61	<i>C. pseudoformosus</i>				GU237071	—	—
62	<i>C. rhodophyllus</i>				HM750925	—	—
63	<i>C. ruber</i>	DDT60	TANZANIA: Iringa	Tibuhwa 1060.2007	<b>JQ976969</b>	<b>JQ976939</b>	<b>JQ976918</b>
64	<i>C. ruber</i>	DDT45	TANZANIA: Kisarawe	Tibuhwa 1045.2007	<b>JQ976966</b>	<b>JQ976936</b>	—
65	<i>C. subalbidus</i>				AY041148	—	—
66	<i>C. subalbidus</i>				AY041150	—	—
67	<i>C. subalbidus</i>				AY041146	—	—
68	<i>C. subalbidus</i>				AY041147	—	—
69	<i>C. subalbidus</i>				AY041149	—	—
70	<i>C. tomentosus</i>	DDT68	TANZANIA: Morogoro	Tibuhwa 1068.2007	<b>JQ976973</b>	<b>JQ976942</b>	<b>JQ976921</b>
71	<i>C. tomentosus</i>	DDT69	TANZANIA: Morogoro	Tibuhwa 1069.2007	<b>JQ976974</b>	<b>JQ976943</b>	<b>JQ976922</b>
72	<i>Cantharellus</i> sp.				HM750917	—	—
73	<i>Cantharellus</i> sp.				HM750922	—	—
74	<i>Cantharellus</i> sp.				HM750928	—	—
75	<i>Cantharellus</i> sp.				HM750930	—	—
76	<i>Cantharellus</i> sp.				HM750926	—	—
77	<i>Cantharellus</i> sp.				HM750918	—	—
78	<i>Cantharellus</i> sp.				HM750921	—	—
79	<i>Cantharellus</i> sp.				AJ271192	—	—
80	<i>Cantharellus</i> sp.				AY041167	—	—
81	<i>Cantharellus</i> sp.				HM750929	—	—
82	<i>Cantharellus</i> sp. 2	DDT70	TANZANIA: Morogoro	Tibuhwa 1070.2007	<b>JQ976975</b>	<b>JQ976944</b>	<b>JQ976923</b>
83	<i>Cantharellus</i> sp. 2	DDT79	TANZANIA: Morogoro	Tibuhwa 1079.2007	<b>JQ976979</b>	<b>JQ976948</b>	<b>JQ976927</b>
84	<i>Clavulina cinerea</i>				AM259211	AF185974	—
	<i>Clavulina</i> sp.						DQ120947
85	<i>Craterellus chantarellus</i> var. <i>intermedius</i>				HM750919	—	—

Table 1. (Continued).

No	Species	Voucher	Locality	Collection no. (UPS)	LSU-GB	5.8-ITS2 GB	ATP6-GB
86	<i>Craterellus cornucopioides</i>				AY700188	—	—
						JF907967	
87	<i>C. cornucopioides</i>				AJ279572	—	—
88	<i>C. lutescens</i>	SS575	SWEDEN: Uppland	Olariaga 2005 (503703)	<b>JQ976982</b>	—	—
89	<i>C. lutescens</i>				EU522746	—	—
90	<i>C. melanoxeros</i>	SS576	SWEDEN: Uppland	Aronsson 2008 (441865)	<b>JQ976983</b>	—	—
91	<i>C. sp.</i>				HM113529	—	—
92	<i>C. tubaeformis</i>				AF287851	—	—
						AF385632	
93	<i>C. tubaeformis</i>	SS572	SWEDEN: Uppland	Lindau 2010	<b>JQ976980</b>	—	—
94	<i>C. tubaeformis</i>				DQ898741	—	—
95	<i>Dacrymyces chrysospermus</i>				AF287855	—	EU339249
96	<i>Hydnum rufescens</i>				AY293187	—	—
97	<i>Multiclavula mucida</i>				AF287875	—	—

of 20 µL. For LSU, and 5.8-ITS2 the PCR thermal cycling parameters were as described in Savić & Tibell (2009) for LSU. Amplification and thermal cycling parameters for PCR of the ATP6 followed, with the modifications, the protocol of Kretzer & Bruns (1999): five cycles of 35 s at 94 °C, 55 s at 37 °C, 1 min at 72 °C, followed by 30 cycles of 35 s at 94 °C, 55 s at 45 °C, and 1 min at 72 °C, and final elongation for 10 min at 72 °C. Amplification products were visualized on 0.5 % agarose gels stained with ethidium bromide and the PCR product was purified using Millipore plates (MultiScreen™ PCR, Danvers, MA). Sequencing, automated reaction clean up, and visualization were carried out as described by MacroGen ([www.macrogen.com](http://www.macrogen.com)).

### Alignments and phylogenetic analyses

To evaluate the phylogenetic relationship in a sample of African taxa, all four data sets (larger dataset of LSU, smaller dataset of LSU, 5.8S-ITS2, and ATP6) were aligned separately using MAFFT (Kato et al. 2002, 2005) on the online server (v. 6), which was used to create alignments that utilized the L-INS-i (for LSU and ATP6) and E-INS-i (5.8-ITS2) MAFFT algorithm. All four alignments were generated using the default settings (gap opening penalty = 1.53 and offset value = 0.00).

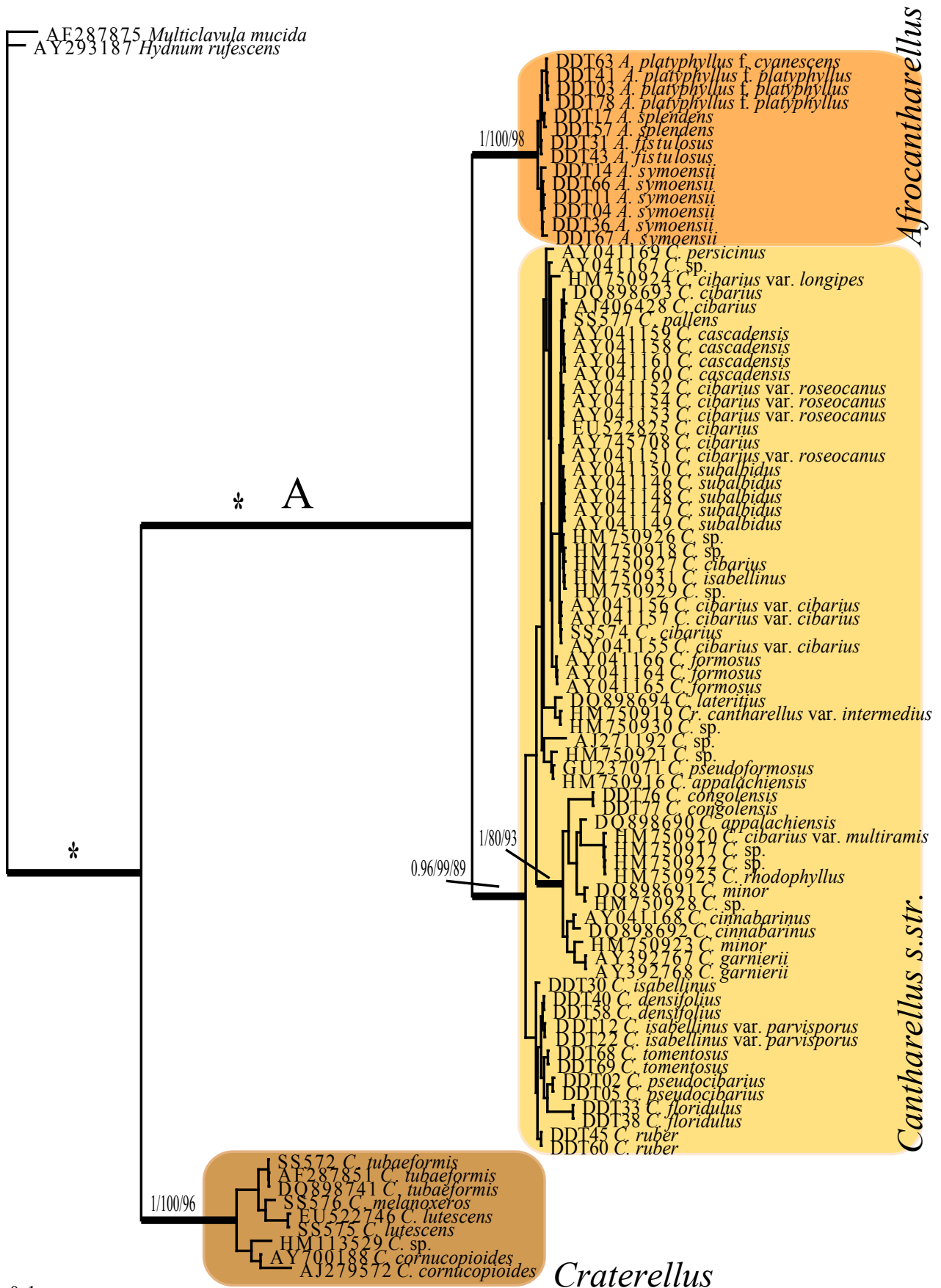
The first LSU dataset was submitted to the Cyberinfrastructure for Phylogenetic Research (CIPRES Science Gateway: <http://www.phylo.org/>) for preliminary analysis with RAxML v. 7.2.8 (Stamatakis 2006, Stamatakis

et al. 2008). Before the final alignment, regions where positional homology was doubtful were excluded from the final alignment.

Using the AIC implemented in JModeltest v. 0.1.1 (Guindon & Gascuel 2003, Posada 2008), the Bayesian analysis employed the GTR+G model for the first dataset (larger LSU matrix), 5.8-ITS2 and ATP6; GTR+G+I was employed for smaller LSU partition (however its likelihood score was also very close to that of the GTR+G model). Before concatenation of the sequences for the second dataset (LSU/5.8-ITS2/ATP6), single-gene analyses were performed to detect significant conflicts among datasets and partitions. A conflict was considered significant if a well-supported monophyletic group, for example MLb  $\geq 70$  % (Mason-Gamer & Kellogg 1996), was found not to be well supported as non-monophyletic when different loci were used. Each single-locus alignment was analyzed separately employing rapid bootstrap heuristics in RAxML v. 7.2.8 (Stamatakis et al. 2008) via a Web server available at the Vital-IT Unit at Swiss Institute of Bioinformatics (<http://phylobench.vital-it.ch/raxml-bb/index.php>), executing 100 rapid bootstrap replicates employing a GTRMIX model (switching from GAMMA to CAT for rapid bootstrapping); thereafter a thorough ML search was conducted under the GAMMA model. No significant incongruence among datasets was detected (data not shown), hence the three matrices were concatenated. After the exclusion of ambiguously aligned regions and introns,

Table 2. Primers used for amplification of the 5.8S-ITS2 part of ITS region.

Primer		Sequence
forward	ITS3C	5'-GCATCGATGAAGAACGCAGT-3'
reverse	Lcan	5'-GTCCGAGTTGTAGATGAG-3'
forward	5.8Scanf	5'-CGATGAAGAACGCAGCG-3'
forward	5canf	5'-CATCGAGTCTTTGAACGCAAAC-3'
reverse	LcanR	5'-ATCGAGTCTTTGAACGCAAAC-3'



**Fig. 2.** Phylogenetic relationships among 92 specimens (Table 1) representing 54 taxa of cantharelloid fungi based on a Bayesian analysis of the large LSU dataset. The tree was rooted using *Multiclavula mucida*. The three support values associated with each internal branch correspond to PP, MPbs and MLb proportions, respectively. Branches in bold indicate a support of PP ≥ 95 % and MPbs, MLb ≥ 70 %. An asterisk on a bold branch indicates that this node has a support of 100 % for all support estimates.



**Table 3.** Morphological features of *Afrocantharellus* and *Cantharellus*.

	<i>Afrocantharellus</i>	<i>Cantharellus</i>
<i>Basidiome colour</i>	always variegated	Mostly uniformly coloured
<i>Hymenophore</i>	well-developed with thick diverging folds	Poorly-developed, without folds or with thin folds but never with thick diverging folds
<i>Folds</i>	thick, blunt, always decurrent and distantly spaced	Relatively thin, sharp, subdecurrent or decurrent and not distantly spaced
<i>Clamp connections</i>	Mostly absent	Mostly present

supported branches, one containing only African species (*Afrocantharellus*; PP=1.0; MPbs=100; MLb=98) and another with both Northern Hemisphere, African, and one New Caledonian species, *Cantharellus s. str.* (PP=0.96; MPbs=99; MLb=89). Species relationships within *Cantharellus s. str.* and *Afrocantharellus* were mostly resolved, although with very low support.

### The combined data set phylogeny

The three-locus Bayesian phylogeny is presented in Fig. 3. *Craterellus*, despite missing ATP6 (the third data set) in the concatenated matrix, was again strongly supported (PP=1.0; MPbs=100; MLb=100) as the sister-group of clade A, *Cantharellus s. lat.* (PP=1.0; MPbs=100; MLb=100). All species in our sampling traditionally placed in *Cantharellus* (*Cantharellus s. lat.*) were recovered as two sister clades, *Cantharellus s. str.* and *Afrocantharellus*, with high support values (PP=1.00, MPbs=97; MLb=96 and PP=1.00, MPbs=100; MLb=93 respectively).

In the phylogenies based on the first and second datasets (large LSU and concatenated LSU/5.8-ITS2/ATP6) *Cantharellus s. lat.* includes two strongly supported subclades, *Cantharellus s. str.* and *Afrocantharellus* for all three support estimates (Figs 2–3).

*Afrocantharellus*, the sister-clade of *Cantharellus s. str.* in both phylogenies obtained high support, and this,

in conjunction with the rather distinctive morphological characteristics of having a well-differentiated hymenophore with diverging folds, the variegated colour of the basidiomes and sometime also the stipe (Table 3, Fig. 4) support the recognition of *Afrocantharellus* at generic level. Based on molecular evidence and morphological features, we suggest emendation revised circumscription of *Cantharellus* to exclude the species closely related to *C. symoensii*, and the elevation of *Cantharellus* subgen. *Afrocantharellus* to generic level.

### TAXONOMY

***Afrocantharellus*** (Eyssart. & Buyck) Tibuhwa, **gen. stat. nov.**

MycoBank MB518687

*Basionym:* *Cantharellus* subgen. *Afrocantharellus* Essyart. & Buyck, *Docums Mycol.* **121**: 55 (2001).

*Type:* *Cantharellus symoensii* Heinem., *Bull. Jard. bot. État Brux.* **36**: 343 (1966).

Basidiomata fleshy, variegated, vividly coloured, red to orange or yellowish, rarely pale; cap 3.5–18 cm diam, hymenophore with very well-differentiated, thick, blunt, distantly spaced and diverging folds, clamp connections mostly absent.

### Key to the species of *Afrocantharellus*

- 1 Basidiomata small to large, cap 3.5–18 cm diam, stipe not compressed laterally, stuffed or solid, clamps absent ..... 2  
Basidiomata small, cap 1.5–2.5 cm diam, stipe laterally compressed and hollow, clamps present ..... 1. **A. fistulosus**
- 2 (1) Basidiomata large and robust, cap 6–18 cm diam; uniformly orange-red; staining hands upon handling; folds yellowish orange; pileipellis a trichoderm ..... 4. **A. splendens**  
Basidiomata medium-sized to large; cap 3.5–12 cm diam; orange-red, but irregularly speckled with other tinges, never staining the hands when handled; folds bright yellow or pale yellow; pileipellis a cutis ..... 3
- 3 (2) Basidiospores ellipsoid (Q = 1.6–2.3); folds bright yellow; cap orange-red, disrupted by pinkish tinges towards the margin ..... 5. **A. symoensii**  
Basidiospores subglobose (Q = 1.2–1.5); folds pale yellowish, no pinkish tinges towards the margin ..... 4
- 4 (3) Stipe, cap margin, and folds with glaucous or bluish tinges ..... 3. **A. platyphyllus f. cyanescens**  
Stipe, cap margin and folds without glaucous or bluish tinges ..... 2. **A. platyphyllus f. platyphyllus**

## Species of *Afrocantharellus*

### 1. *Afrocantharellus fistulosus* (Tibuhwa & Buyck)

Tibuhwa, **comb. nov.**

MycoBank MB800280

(Fig. 4B)

*Basionym:* *Cantharellus fistulosus* Tibuhwa & Buyck, *Cryptogamie, Mycol.* **29**: 133 (2008).

*Type: Tanzania: Coast region*, Kazimzumbwi forest reserve, Kisarawe, 06°04'32" S, 039°15'56" E, miombo dominated by *Brachystegia*, *Combretum* and *Julbernardia*, April 2007, *Tibuhwa D 43.2007* (UPS – holotype; isotypes: PC, UDSM – isotypes).

*Description:* Tibuhwa *et al.* (2008).

*Distribution:* Known only from Tanzania.

*Comments:* This species is easily recognized in the field by its small size, yellow colour, cap with clearly brown matted centre, pink hymenophore composed of widely spaced folds, and by the smooth hollow stipe, which is slightly twisted or compressed.

*Other material examined: Tanzania: Coast region:* Kazimzumbwi forest reserve, Kisarawe, 06°04'32" S, 039°15'56" E, *Tibuhwa D 31.2006* (UPS, UDSM). *Iringa region:* Madibira forest, 08°15'08" S and 35°17'21" E, alt. 1847 m, in Uapaca woodland, May 2007, *Tibuhwa D 59.2007* (UPS, UDSM).

### 2. *Afrocantharellus platyphyllus* (Heinem.) Tibuhwa, **comb. nov. f. platyphyllus**

MycoBank MB518693

*Basionym:* *Cantharellus platyphyllus* Heinem., *Bull. Jard. bot. État Brux.* **36**: 342 (1966).

*Type: Democratic Republic of Congo:* Elisabethville, 1932, *De Loose 31* (BR – holotype).

*Vernacular names:* Tanzania (Bena dialect): Bunyamalagata, Wifindi (Hehe dialect): Wisogolo.

*Basidiomata* medium-sized to large. *Cap* 3.5–9.5 cm wide, deep orange crimson towards the cap centre. *Folds* well-developed, yellow, thick and distantly spaced, forking or with numerous cross-veins. *Stipe* 1.5–6.5 × 1–1.5 cm, solid, slightly attenuated toward the base and pale yellow in colour. *Basidia* clavate (44.1–)55.4(–70.0) × (5.2–)7.2(–9.2) µm (Q = 6.6–9.2). *Basidiospores* subglobose, (6.3–)7.5(–8.6) × (5.0–)6.2(–7.1) µm (Q = 1.1–1.5). *Suprapellis* a cutis of 10–12 µm wide hyphae. *Clamps* none.

*Distribution:* Reported from Burundi (Buyck 1994), the Democratic Republic of Congo (Heineman 1966), Tanzania (Härkönen *et al.* 1995, Buyck *et al.* 2000), and Zimbabwe (Sharpe & Wursten, <http://www.vumba-nature.com>).

*Comments:* This species is quite distinct in the deep orange to crimson colour, especially towards the cap centre, which

clearly contrasts with the pale bright yellow folds. In the field it resembles *A. symoensii* but lacks the pink tinge on the basidiomes of *A. symoensii*; it also differs in subglobose basidiospores, rather than the ellipsoid ones of *A. symoensii*.

*Descriptions and illustrations:* Heinemann (1966) and Härkönen *et al.* (1995, 2003).

*Other material examined: Tanzania: Coast region:* Kisarawe, 06°04'32" S, 039°15'48" E, *Tibuhwa 1041.2006* (UPS, UDSM); *Morogoro region:* SUA forest reserve, 06°52'34" S, 37°67'29" E, *Tibuhwa 1003.2004* (UPS, PC, UDSM); *Iringa region:* Madibira forest, 08°15'08" S, 35°17'21" E, *Tibuhwa 1078.2007* (UPS, UDSM); Vigama village, *Buyck 98.126* (PC), *Buyck 98.127* (PC), *Buyck 98.130* (PC).

### 3. *Afrocantharellus platyphyllus* f. *cyanescens* (Buyck) Tibuhwa, **comb. nov.**

MycoBank MB518693

(Fig. 4D)

*Basionym:* *Cantharellus cyanescens* Buyck, *Ubwoba: Champ. Comest. l'Ouest Burundi* [Publ. Agricole no. 34]: 112 (1994).

*Type: Burundi:* Nyamirambo, 1994, *Buyck* (BR – holotype).

*Vernacular names:* Tanzania (Hehe dialect): Wisogolo; (Bena dialect): Wifindi, Bunyamalagata. Burundi (Kirundi dialect): Peri Itukura.

*Basidiomata* medium-sized to large. *Cap* 5–10 cm wide, in the field with conspicuous glaucous or bluish tinges on the orange-red cap, margin and folds especially in young stages, but later fading. *Folds* deeply decurrent, thick, blunt, diverging, distantly spaced, strongly meshed, bright yellow speckled with bluish grey tinges. *Stipe* 3–6 × 0.9–1.3 cm, smooth, solid, cylindrical, the same colour as the folds in the upper half while fading to grey-cream towards the base. *Basidia* clavate (45.0–)55.0(–75.0) × (5.0–)7.0(–7.5) µm (Q = 6.3–9.8), with 2–4 spores. *Basidiospores* (7.5–)10.0(–10.6) × (5.2–)6.1(–6.5) µm (Q = 1.3–1.5), smooth, broadly ellipsoid to subglobose. *Suprapellis* a cutis of 8.0–15 µm wide hyphae. *Clamps* none.

*Distribution:* Burundi (Buyck 1994) and Tanzania (newly reported here).

*Comments:* This taxon is recognized in the field by its fleshy deep orange cap interrupted by blue or glaucous tinges and folds which are strongly meshed and not purely yellow but with orange–grey tinges. These unique tinges on the cap, stipe and folds distinguish it from the otherwise very similar *A. platyphyllus* f. *platyphyllus*.

*Description:* Buyck (1994).

*Other material examined: Tanzania: Morogoro region:* Ubenazomosi woodland, 06°55'11" S, 037°35'20" E, *Tibuhwa 1063.2007* (UPS, UDSM), *Tibuhwa 1056.2007* (UPS, UDSM); *Coast region:* Kisarawe, 06°04'32" S, 039°15'56" E, *Tibuhwa 1034.2006* (UPS, UDSM).



**Fig. 4.** Basidiomes of *Afrocantharellus* and *Cantharellus* species showing morphological differences of the hymenophores: **A.** *Afrocantharellus symoensii* (Tibuhwa 1011.2005; UPS). **B.** *A. fistulosus* (holotype). **C.** *A. splendens* (DDT 1053.2011; UDSM). **D.** *A. platyphyllus* f. *cyanescens* (Tibuhwa 1063.2007; UPS). **E.** *Cantharellus congolensis* (Tibuhwa 1076.2007; UDSM). **F.** *C. rufopunctatus* (Tibuhwa 1010.2004; UDSM). All photos taken in Tanzania by Donatha D. Tibuhwa.

**4. *Afrocantharellus splendens* (Buyck) Tibuhwa, comb. nov.**

Mycobank MB518692

(Fig. 4C)

*Basionym:* *Cantharellus splendens* Buyck, *Ubwoba: Champ. Comest. l'Ouest Burundi* [Publ. Agricole no. 34]: 112 (1994).

*Type:* **Burundi:** under *Brachystegia*, *Buyck 5518* (BR – holotype).

*Vernacular names:* Tanzania (Nyambo dialect): Binyantuku. Burundi (Kirundi dialect): Peri magufa.

*Basidiomata* large. *Cap* 8–18 cm wide, bright orange-red. *Folds* thick, blunt diverging, distantly spaced, pale yellow with orange tinges. *Stipe* 2.5–7 × 1.2–3.5 cm, smooth, solid, subcylindrical, slightly attenuated toward the base, of the same colour as the cap but paling to white toward the base. *Basidia* narrowly cylindrical-clavate, (40.0–)49.7(–57.4) × (5.4–)6.6(–7.7) μm (Q = 6.7–9.1). *Basidiospores* ellipsoid (8.1–)9.9(–12.0) × (3.7–)4.2(–4.7) μm (Q = 2.0–2.7). *Suprapellis* a trichoderm of more or less ramified, hyphae 5.5–8.0 μm wide. *Clamps* none.

*Distribution*: Burundi (Buyck 1994), and Tanzania (Buyck *et al.* 2000).

*Comments*: This species is easily recognized in the field by the large, fleshy and bright orange-red basidiomes, which recall those of *A. symoensii* and *A. platyphyllus*. The pigmentation of the cap stains the hands upon handling, and microscopically a trichoderm pileipellis distinguishes it from these other two species.

*Description*: Buyck (1994).

*Other material examined*: **Tanzania**: *Morogoro region*: Ubenazomosi woodland, 06°55'11" S, 037°34'20" E, *Tibuhwa 1057.2007* (UPS, UDSM); *Mwanza region*: Geita-Rwamgasa forest reserve, 03°09'50" S, 32°04'52" E, *Tibuhwa 1017.2005* (UPS, UDSM).

## 5. *Afrocantharellus symoensii* (Heinem.) Tibuhwa, **comb. nov.**

MycoBank MB518691

(Fig. 4A)

*Basionym*: *Cantharellus symoensii* Heinem., *Bull. Jard. bot. État. Brux.* **36**: 343 (1966).

*Type*: **Democratic Republic of Congo**: Kasumbalesa, 1958, *Symoens 6037* (BR – holotype).

*Vernacular names*: Tanzania (Nyamwezi dialect): Mkukwe. (Bena dialect): Wifindi, (Hehe dialect): Wisogolo. Burundi (Kirundi dialect): Peri nyakeke, Peri itukura.

*Basidiomata* medium-sized to large. *Cap* 3.5–8 cm wide, smooth, orange-red disrupted with pale pink and yellow patches especially towards the margin. *Folds* thick, blunt, diverging, distantly spaced, yellow or slightly pale. *Stipe* 2.5–4 × 0.9–2 cm, smooth, solid or rarely somewhat lax at maturity, cylindrical but slightly wider towards the cap, of the same colour as the folds. *Basidia* clavate (38.2–)48.7(–59.3) × (5.0–)6.5(–8) μm (Q = 6.3–10.0). *Basidiospores* (7.4–)9.0(–10.6) × (4.5–)4.9(–5.2) μm (Q = 1.6–2.3), ellipsoid. *Suprapellis* a cutis of 7.5–10 μm wide hyphae. *Clamps* none.

*Distribution*: Reported from Burundi (Buyck 1994), the Democratic Republic of Congo (Heineman 1966), Tanzania (Buyck *et al.* 2000, Härkönen *et al.* 1995), and Zambia (Eyssartier & Buyck 1998).

*Comments*: This is one of the most common *Afrocantharellus* species in tropical Africa. It is easily recognized in the field

by the fleshy orange-red cap with yellow and pink patches towards the margin, and the bright yellow, distantly spaced, thick folds. It has often been confounded with *C. longisporus*, but differs in the differently shaped spores, and in lacking clamp connections (Eyssartier & Buyck 1998, Buyck *et al.* 2000).

*Descriptions and illustrations*: Eyssartier & Buyck (1998) give a detailed description of the holotype, and more descriptions and/or illustration are found in Buyck (1994), Heinemann (1966), and Härkönen *et al.* (1995, 2003).

*Other material examined*: **Tanzania**: *Morogoro region*: SUA forest reserve, 06°51'22" S, 37°39'23" E, *Tibuhwa 1004.2005* (UPS, PC, UDSM); Ubenazomosi woodland, 06°55'11" S, 37°34'20" E, *Tibuhwa 1011.2005* (UPS, PC, UDSM); *Coast region*: Kazimzumbwi forest reserve, S 06°04'32" S, 039°15'56" E, *Tibuhwa 1007.2005* (UPS, PC, UDSM), *Tibuhwa 1036.2005* (UPS, UDSM), *Tibuhwa 1037.2006* (UPS, UDSM); *Mwanza region*: Geita-Polepole forest reserve, 02°52'29" S, 32°07'27" E, *Tibuhwa 1014.2004* (UPS, PC, UDSM); *Tabora region*: Masange forest reserve, 04°59'22" S, 032°40'20" E, *Tibuhwa 1021.2005* (UPS, UDSM); *Iringa region*: Madibira forest, *Tibuhwa 1067.2007* (UPS, UDSM), *Tibuhwa 1066.2007* (UPS, UDSM); *Dar e Salaam District*: bought in a market, *Buyck 98.113* (PC, UDSM); *Coast region*: Msanga area, near Chanika village, *Buyck 98.011* (PC, UDSM).

## DISCUSSION

There are no major strongly supported species group subclades in the LSU- phylogeny of *Cantharellus s. str.*, except for a well-supported clade containing *Cantharellus congolensis* (PP=1.00; MPbs=80; MLb=93) that almost exclusively (apart from *C. congolensis* and *C. garnierii*) contains Northern Hemisphere species. *Cantharellus congolensis* (Fig. 4E) was placed in subgen. *Afrogomphus* by Eyssartier & Buyck (2001), and *C. floridulus*, which was placed in subgen. *Rubrinus* (Eyssartier & Buyck 2001), have relatively long branch-lengths, but with low support. That the name of the generic type species, *C. cibarius*, is present on several subclades in the LSU analysis of *Cantharellus s. str.* supports the opinion that this name may either embrace several cryptic species, or that many morphologically similar species and infraspecific taxa have been included under that name. Only by combining extensive molecular data with critical morphological studies will further elucidate the taxonomy and systematics of this group.

*Afrocantharellus* was a strongly supported clade in the LSU phylogeny (Fig. 2) with only a limited variation among the species in the LSU region investigated. *Afrocantharellus* is, however, strongly supported in the three-gene phylogeny (Fig. 3) and species are reasonably well resolved, the only exception being *A. splendens*. For both specimens of *A. splendens* (DDT17 and DDT57) we managed to obtain all three regions (LSU/5.8-ITS2/ATP6), with ATP6 being slightly shorter in one, however, *A. splendens* is monophyletic in the large LSU phylogeny (Fig. 2).

*Afrocantharellus*, as represented recently by *C. platyphyllus* and *C. symoensii* in a one-gene phylogeny (tef-

1) by Buyck & Hofstetter (2011) and Buyck *et al.* (2011), the clade was also distinct. In this phylogeny, which was basically the same in both papers, the systematic arrangement follows Eyssartier & Buyck (2001) although there was no support in the phylogeny for the lower branches. This might be due to the *tef-1* seeming to be a slow-evolving gene, for example in comparison to *RPB2* (Matheny *et al.* 2007). In our LSU phylogeny (Fig. 2), *C. fistulosus* is within *Afrocantharellus*. Although recently described from Tanzania as *Cantharellus fistulosus* (Tibuhwa *et al.* 2008), and also morphologically reported as best fitting in subgenus *Parvocantharellus* as defined by Eyssartier & Buyck (2001) and based on characters such as the abundance of clamp connections. However, molecular data place this species in *Afrocantharellus*, and thus the absence of clamp connection is not a synapomorphy for *Afrocantharellus*. The species of *Afrocantharellus* are morphologically reasonably well-characterized (Table 3), and a short description of the species is given in the taxonomic part above. It consists of species closely related to *A. symoensii*, e.g. *A. platyphyllus* f. *platyphyllus*, which in the field is difficult to distinguish from *A. symoensii*. Other taxa included are *A. platyphyllus* f. *cyanescens*, *A. splendens*, and *A. fistulosus*. Eyssartier & Buyck (2001) referred these species to *Cantharellus* subgen. *Afrocantharellus*, except for *A. fistulosus*. However, *Afrocantharellus* is characterized by having a well-differentiated hymenophore with diverging folds, and all species apart from *A. fistulosus* lack clamp connections.

Relying only on morphological characters may be misleading in the study of these difficult taxa (Buyck & Hofstetter 2011, Buyck *et al.* 2011). It was obvious in our analyses that some species names used for sequences in GenBank had been misapplied, such as *Cantharellus cibarius* and *C. minor*. Combining morphological and molecular data, is clearly the best approach to make progress in the study of genera with a rather uniform morphology where few characters are available for morphological study. Moreover, that we do not have clear morphological synapomorphies for all monophyletic groups within former *Cantharellus s. lat.* should not discourage the recognition of further taxa in the future.

## ACKNOWLEDGEMENTS

We are grateful to Sida-SAREC, through the International Science Programme at Uppsala University and the Molecular Biology project of the University of Dar es Salaam, for financial support. We are also indebted to the curators of BR, PC, and UPS for placing material at our disposal. We are further grateful to Bart Buyck for some specimen identifications, and also wish to express our gratitude to two anonymous reviewers, and the helpful work of the editors.

## REFERENCES

- Bas C (1969) Morphology and subdivision of *Amanita* and a monograph on its section *Lepidella*. *Persoonia* **5**: 285–579.
- Binder M, Hibbett DS (2002) Higher-level phylogenetic relationships of *Homobasidiomycetes* (mushroom-forming fungi) inferred from four rDNA regions. *Molecular Phylogenetics and Evolution* **22**: 76–90.
- Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G (2005) The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (*Homobasidiomycetes*). *Systematics and Biodiversity* **3**: 113–157.
- Buyck B (1994) *Ubwoba: Les Champignons Comestibles de l'Ouest du Burundi*. [Publication Agricole no. 34.] Bruxelles: Administration Generale de la Cooperation au Developpement.
- Buyck B, Nzigidahera B (1995) Ethnomycological notes from western Burundi. *Belgian Journal of Botany* **128**: 131–138.
- Buyck B, Hofstetter V (2008) A multigene phylogeny for worldwide *Cantharellus*. *Inoculum* **59**: 22.
- Buyck B, Hofstetter V (2011) The contribution of *tef-1* sequences to species delimitation in the *Cantharellus cibarius* complex in southeastern USA. *Fungal Diversity* **49**: 35–46.
- Buyck B, Cruaud C, Couloux A, Hofstetter V (2011) *Cantharellus texensis* sp. nov. from Texas, a southern lookalike of *C. cinnabarinus* revealed by *tef-1* sequence data. *Mycologia* **103**: 1037–1046.
- Buyck B, Eyssartier G, Kivaisi A (2000) Addition to the inventory of the genus *Cantharellus* (*Basidiomycota*, *Cantharellaceae*) in Tanzania. *Nova Hedwigia* **71**: 3–4.
- Carriconde F, Gardes M, Jargeat P, Heilmann-Clausen J, Mouhamadou B, Gryta H (2008) Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, *Tricholoma scalpturatum*. *Microbial Ecology* **56**: 513–524.
- Dahlman M, Danell E, Spatafora JW (2000) Molecular systematics of *Craterellus*: cladistic analyses of nuclear LSU rDNA sequence data. *Mycological Research* **104**: 388–394.
- Dunham SM, Kretzer A, Pfrender ME (2003) Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. *Molecular Ecology* **12**: 1607–1618.
- Eyssartier G (2003) *The genus Cantharellus*. PhD thesis, Muséum National d'Histoire Naturelle Paris.
- Eyssartier G, Buyck B (1998) Contribution à la systématique du genre *Cantharellus* en Afrique tropicale: étude de quelques espèces rouges. *Belgian Journal of Botany* **131**: 139–149.
- Eyssartier G, Buyck B (2001) Nomenclatural and systematic note on the genus *Cantharellus*. *Documents Mycologiques* **121**: 55–56.
- Eyssartier G, Buyck B, Courtecuisse R (2001) New species and combinations in cuboid-spored *Entoloma* species from Madagascar. *Mycological Research* **105**: 1144–1148.
- Eyssartier G, Stubbe D, Walleyn R, Verbeke A (2009) New records of *Cantharellus* species (*Basidiomycota*, *Cantharellaceae*) from Malaysian dipterocarp rainforest. *Fungal Diversity* **36**: 57–67.
- Feibelman TP, Bayman P, Cibula WG (1994) Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research* **98**: 614–618.
- Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the *Cantharellaceae* inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research* **101**: 1423–1430.
- Frøslev TG, Jeppesen TS, Læssøe T, Kjeller R (2007) Molecular phylogenetics and delimitation of species in *Cortinarius* section *Calochroi* (*Basidiomycota*, *Agaricales*) in Europe. *Molecular Phylogenetics and Evolution* **44**: 217–227.
- Geml J, Laursen GA, O'Neill K, Nusbaum HC, Taylor DL (2006)

- Beringian origins and cryptic speciation events in the Fly Agaric (*Amanita muscaria*). *Molecular Ecology* **15**: 225–239.
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.
- Härkönen M, Niemelä T, Mwasumbi L (1995) Edible mushrooms of Tanzania. *Karstenia* **35** (Suppl.): 1–92.
- Härkönen M, Niemelä T, Mwasumbi L (2003) Tanzanian mushrooms: edible, harmful and other fungi. *Norrinia* **10**: 1–200.
- Heinemann P (1958) Champignons récoltes au Congo Belge par Madame Gossens-Fontana III. *Cantharellineae. Bulletin du Jardin Botanique de l'Etat Bruxelles* **28**: 385–438.
- Heinemann P (1966) Cantharellineae du Katanga. *Bulletin du Jardin Botanique de l'Etat Bruxelles* **36**: 335–352.
- Henkel TW, Meszaros R, Catherine AM, Kennedy A (2005) New *Clavulina* species from the Pakaraima Mountains of Guyana. *Mycological Progress* **4**: 343–350.
- Hey J (2006) On the failure of modern species concepts. *Trends in Ecology & Evolution* **21**: 447–450.
- Hibbett DS, Donoghue MJ (2001) Analysis of character correlations among wood decay mechanisms, mating systems, and substrate ranges in homobasidiomycetes. *Systematic Biology* **50**: 215–242.
- Hibbett DS, Gilbert LB, Donoghue MJ (2000) Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407**: 506–508.
- Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proceedings of the National Academy of Sciences, USA* **94**: 12002–12006.
- Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* **33**: 511–518.
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–3066.
- Kretzer AM, Bruns TD (1999) Use of atp6 in fungal phylogenetics: an example from the *Boletales*. *Molecular Phylogenetics and Evolution* **13**: 483–492.
- Larsson KH, Larsson E, Kõljalg U (2004) High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research* **108**: 983–1002.
- Mason-Gamer R, Kellogg E (1996) Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae* (*Graminae*). *Systematic Biology* **45**: 524–545.
- Matheny PB (2005) Improving phylogenetic inference of mushrooms with *RPB1* and *RPB2* nucleotide sequences (*Inocybe*, *Agaricales*). *Molecular Phylogenetics and Evolution* **35**: 1–20.
- Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer GE, McLaughlin DJ, Wilson AW, Frøslev T, Ge ZW, Kerrigan RW, Slot JC, Vellinga EC, Liang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vaura J, Hibbett DS (2007) Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (*Basidiomycota*, *Fungi*). *Molecular Phylogenetics and Evolution* **43**: 430–51.
- Moncalvo JM, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, McLenon T, Margaritescu S, Weiß M, Garnica S, Danell E, Langer G, Langer E, Larsson E, Larsson KH, Vilgalys R (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. *Mycologia* **98**: 937–948.
- Nagy LG, Házi J, Vágvölgyi C, Papp T (2012) Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species. *Mycologia* **104**: 254–275.
- Olariaga I, Jugo BM, García-Etxebarria K, Salcedo I (2009) Species delimitation in the European species of *Clavulina* (*Cantharellales*, *Basidiomycota*) inferred from phylogenetic analyses of ITS region and morphological data. *Mycological Research* **113**: 1261–1270.
- Pilz D, Norvell L, Danell E, Molina R (2003) *Ecology and Management of Commercially Harvested Chanterelle Mushrooms*. [USDA Forest Service Technical Report no. PNW-GTR-576.] Portland, OR: Pacific Northwest Research Station, USDA Forest Service.
- Pine EM, Hibbett DS, Donoghue MJ (1999) Phylogenetic relationship of cantharelloid and clavarioid homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia* **91**: 944–963.
- Posada D (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* **25**: 1253–1256.
- Ronquist F, Huelsenbeck JP (2005) *MrBayes*. Version 3.1 (Bayesian analysis of phylogeny); [www.mrbayes.net](http://www.mrbayes.net)
- Savić S, Tibell L (2009) Taxonomy and species delimitation in *Sporodictyon* (*Verrucariaceae*) in Northern Europe and the adjacent Arctic—reconciling molecular and morphological data. *Taxon* **58**: 585–605.
- Shao S-C, Tian X-F, Liu P-G (2011) *Cantharellus* in southwestern China: a new species and a new record. *Mycotaxon* **116**: 437–446.
- Sharpe C, Wursten B (2009) *A preliminary list of the macrofungi recorded in the Vumba*. [www.vumba-nature.com](http://www.vumba-nature.com).
- Stamatakis A (2006) RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Stamatakis A, Hoover P, Rougemont J (2008) A Rapid Bootstrap algorithm for the RAXML Web-Servers. *Systematic Biology* **75**: 758–771.
- Swofford DL (2002) *PAUP: phylogenetic analysis using parsimony (and other methods)*. Version 4.0b10. Sunderland, MA: Sinauer Associates.
- Thacker JR, Henkel TW (2004) New species of *Clavulina* from Guyana. *Mycologia* **96**: 650–657.
- Tibuhwa D, Buyck B, Kivaisi A, Tibell L (2008) *Cantharellus fistulosus* sp. nov. from Tanzania. *Cryptogamie Mycologie* **29**: 129–135.
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.