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

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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

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, Tibuhwa 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. symoensis 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 Tulasiella. 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 chanterelloid clade. This, however, primarily had a background in incongruent placement of Tulasiella with different datasets, whereas LSU still seems to efficiently resolve relationships, also in Botryobasidium and Tulasiella. Problems in using LSU datasets include long-branch attraction in some types of analyses, particularly in distance and parsimony-based analyses (Moncalvo et al.
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. Carron 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 infragenetic taxa, as monophyletic groups, is not supported (e.g. Geml et al. 2006, Freslev 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, 5.8S-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 – AV = max-SD (max) Q, in which min = lowest value recorded for the measured feature, max = highest value, AV = arithmetic mean and SD standard deviation; Q the ratio length/width (Eysartier 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
Afrocantharellus gen. nov.

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/phylows/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 (VIOPEN). 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 µL diluted or undiluted DNA, 1.5 µL of each primer (10 µM), 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  | *Afrocantharellus fistulosus*                | DDT31   | TANZANIA: Kisarawe           | Tibuha 31.2006       | JQ976959| —           | —       |
| 2  | A. fistulosus                                | DDT43   | TANZANIA: Kisarawe           | Tibuha 43.2007       | JQ976965| —           | —       |
| 3  | A. platyphyllus                              | DDT63   | TANZANIA: Morogoro           | Tibuha 1063.2007     | JQ976970| —           | —       |
|    | f. cyanescens                               |         |                               |                       |         |             |         |
| 4  | A. platyphyllus                              | DDT78   | TANZANIA: Iringa             | Tibuha 1078.2007     | JQ976978| JQ976947    | JQ976926|
|    | f. platyphyllus                             |         |                               |                       |         |             |         |
| 5  | A. platyphyllus                              | DDT03   | TANZANIA: Morogoro           | Tibuha 1003.2004     | JQ976950| JQ976929    | —       |
|    | f. platyphyllus                             |         |                               |                       |         |             |         |
| 6  | A. platyphyllus                              | DDT41   | TANZANIA: Kisarawe           | Tibuha 1041.2006     | JQ976964| —           | —       |
|    | f. platyphyllus                             |         |                               |                       |         |             |         |
| 7  | A. splendens                                 | DDT57   | TANZANIA: Morogoro           | Tibuha 1057.2007     | JQ976967| JQ976937    | JQ976916|
| 8  | A. splendens                                 | DDT17   | TANZANIA: Geita              | Tibuha 1017.2005     | JQ976956| JQ976932    | JQ976911|
| 9  | A. symoensii                                | DDT36   | TANZANIA: Kisarawe           | Tibuha 1036.2005     | JQ976961| JQ976934    | JQ976914|
| 10 | A. symoensii                                | DDT04   | TANZANIA: Morogoro           | Tibuha 1004.2005     | JQ976951| —           | —       |
| 11 | A. symoensii                                | DDT66   | TANZANIA: Iringa             | Tibuha 1066.2007     | JQ976971| JQ976940    | JQ976919|
| 12 | A. symoensii                                | DDT11   | TANZANIA: Morogoro           | Tibuha 1011.2005     | JQ976953| —           | —       |
| 13 | A. symoensii                                | DDT67   | TANZANIA: Iringa             | Tibuha 1067.2007     | JQ976972| JQ976941    | JQ976920|
| 14 | A. symoensii                                | DDT14   | TANZANIA: Geita              | Tibuha 1014.2004     | JQ976955| —           | —       |
| 15 | Botryobasidium isabellinum                  |         |                               |                       |         |             | —       |
| 16 | C. appalachiensis                           |         |                               |                       |         |             | —       |
| 17 | C. appalachiensis                           |         |                               |                       |         |             | —       |
| 18 | C. cascadensis                              |         |                               |                       |         |             | —       |
| 19 | C. cascadensis                              |         |                               |                       |         |             | —       |
| 20 | C. cascadensis                              |         |                               |                       |         |             | —       |
| 21 | C. cascadensis                              |         |                               |                       |         |             | —       |
| 22 | C. cibarius var. cibarius                   |         |                               |                       |         |             | —       |
| 23 | C. cibarius var. cibarius                   |         |                               |                       |         |             | —       |
| 24 | C. cibarius var. cibarius                   |         |                               |                       |         |             | —       |
| 25 | C. cibarius var. roseocanus                 |         |                               |                       |         |             | —       |
| 26 | C. cibarius var. roseocanus                 |         |                               |                       |         |             | —       |
| 27 | C. cibarius var. roseocanus                 |         |                               |                       |         |             | —       |
| 28 | C. cibarius var. roseocanus                 |         |                               |                       |         |             | —       |
| 29 | C. cibarius var. multiramis                 |         |                               |                       |         |             | —       |
| 30 | C. cibarius                                | SS574   | SWEDEN: Uppland              | Olariaga & Felipe 2005/503752 | JQ976981| —           | —       |
| 31 | C. cibarius                                |         |                               |                       |         |             | —       |
| 32 | C. cibarius                                |         |                               |                       |         |             | —       |
| 33 | C. cibarius                                |         |                               |                       |         |             | —       |
| 34 | C. cibarius                                |         |                               |                       |         |             | —       |
| 35 | C. cibarius                                |         |                               |                       |         |             | —       |
| 36 | C. cibarius var. longipes                  |         |                               |                       |         |             | —       |
| 37 | C. cinnabarinus                            |         |                               |                       |         |             | —       |
| 38 | C. cinnabarinus                            |         |                               |                       |         |             | —       |
| 39 | C. congolensis                              | DDT77   | TANZANIA: Morogoro           | Tibuha 1077.2007     | JQ976977| JQ976946    | JQ976925|
| 40 | C. congolensis                              | DDT76   | TANZANIA: Iringa             | Tibuha 1076.2007     | JQ976976| JQ976945    | JQ976924|
| No | Species | Voucher | Locality | Collection no. (UPS) | LSU-GB   | 5.8-ITS2 GB | ATP6-GB |
|----|---------|---------|----------|---------------------|----------|-------------|---------|
| 41 | C. densifolius | DDT40 | TANZANIA: Kisarawe | Tibuhwa 1040.2006 | JQ976963 | JQ976935 | JQ976915 |
| 42 | C. densifolius | DDT58 | TANZANIA: Morogoro | Tibuhwa 1058.2006 | JQ976968 | JQ976938 | JQ976917 |
| 43 | C. floridulus | DDT33 | TANZANIA: Morogoro | Tibuhwa 1033.2006 | JQ976980 | — | JQ976913 |
| 44 | C. floridulus | DDT38 | TANZANIA: Morogoro | Tibuhwa 1038.2005 | JQ976962 | — | — |
| 45 | C. formosus | — | — | — | — | — | — |
| 46 | C. formosus | — | — | — | — | — | — |
| 47 | C. formosus | — | — | — | — | — | — |
| 48 | C. garnieri | — | — | — | — | — | — |
| 49 | C. garnieri | — | — | — | — | — | — |
| 50 | C. isabellinus | — | — | — | — | — | — |
| 51 | C. isabellinus | DDT30 | TANZANIA: Morogoro | Tibuhwa 1030.2006 | JQ976958 | — | — |
| 52 | C. isabellinus var. parvisporus | DDT12 | TANZANIA: Morogoro | Tibuhwa 1012.2004 | JQ976954 | JQ976931 | JQ976910 |
| 53 | C. isabellinus var. parvisporus | DDT22 | TANZANIA: Geita | Tibuhwa 1022.2005 | JQ976957 | JQ976933 | JQ976912 |
| 54 | C. lateritius | — | — | — | — | — | — |
| 55 | C. minor | — | — | — | — | — | — |
| 56 | C. minor | — | — | — | — | — | — |
| 57 | C. pallens | SS577 | SWEDEN: Uppland | Danell & Oliariaga 2005 (503727) | JQ976984 | — | — |
| 58 | C. persiciinus | — | — | — | — | — | — |
| 59 | C. pseudocibarius | DDT02 | TANZANIA: Morogoro | Tibuhwa 1002.2004 | JQ976949 | JQ976928 | JQ976908 |
| 60 | C. pseudocibarius | DDT05 | TANZANIA: Geita | Tibuhwa 1005.2004 | JQ976952 | JQ976929 | JQ976909 |
| 61 | C. pseudoformosus | — | — | — | — | — | — |
| 62 | C. rhodophyllus | — | — | — | — | — | — |
| 63 | C. ruber | DDT60 | TANZANIA: Iringa | Tibuhwa 1060.2007 | JQ976969 | JQ976939 | JQ976918 |
| 64 | C. ruber | DDT45 | TANZANIA: Kisarawe | Tibuhwa 1045.2007 | JQ976966 | JQ976936 | — |
| 65 | C. subalbidus | — | — | — | — | — | — |
| 66 | C. subalbidus | — | — | — | — | — | — |
| 67 | C. subalbidus | — | — | — | — | — | — |
| 68 | C. subalbidus | — | — | — | — | — | — |
| 69 | C. subalbidus | — | — | — | — | — | — |
| 70 | C. tomentosus | DDT68 | TANZANIA: Morogoro | Tibuhwa 1068.2007 | JQ976973 | JQ976942 | JQ976921 |
| 71 | C. tomentosus | DDT69 | TANZANIA: Morogoro | Tibuhwa 1069.2007 | JQ976974 | JQ976943 | JQ976922 |
| 72 | Cantharellus sp. | — | — | — | — | — | — |
| 73 | Cantharellus sp. | — | — | — | — | — | — |
| 74 | Cantharellus sp. | — | — | — | — | — | — |
| 75 | Cantharellus sp. | — | — | — | — | — | — |
| 76 | Cantharellus sp. | — | — | — | — | — | — |
| 77 | Cantharellus sp. | — | — | — | — | — | — |
| 78 | Cantharellus sp. | — | — | — | — | — | — |
| 79 | Cantharellus sp. | — | — | — | — | — | — |
| 80 | Cantharellus sp. | — | — | — | — | — | — |
| 81 | Cantharellus sp. | — | — | — | — | — | — |
| 82 | Cantharellus sp. 2 | DDT70 | TANZANIA: Morogoro | Tibuhwa 1070.2007 | JQ976975 | JQ976944 | JQ976923 |
| 83 | Cantharellus sp. 2 | DDT79 | TANZANIA: Morogoro | Tibuhwa 1079.2007 | JQ976979 | JQ976948 | JQ976927 |
| 84 | Clavulina cinerea | — | — | — | — | — | — |
| 85 | Craterellus chantarellus. var. intermedius | — | — | — | — | — | — |
Table 1. (Continued).

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

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

| Primer | Sequence                  |
|--------|---------------------------|
| forward | 5’–GCATCGATGAAGAAGCGCAGT–3’ |
| reverse | 5’–GTCGAGTTGATGAG–3’       |
| forward | 5’–CGATGAGAAGCAGCGACG–3’   |
| forward | 5’–CATCGAGTCTTTGAACGCAAAC–3’ |
| reverse | 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.
the concatenated data matrix contained 1906 unambiguously aligned sites.

Phylogenetic relationships were inferred separately for both data sets, the first larger LSU dataset and the second concatenated LSU/5.8-ITS2/ATP6 dataset, based on Bayesian analysis. Using MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2005) for each analysis two parallel runs were carried out for two million generations. Each run included four chains, and trees were sampled every 100 generations; we stopped the runs when the average standard deviation of split frequencies (across different runs) was ≤ 0.01. Using relative burn-in the first 25 % of sampled trees were discarded.

In order to obtain additional support values, Maximum parsimony (MP) analyses as well as MP bootstrapping (MPbs) of both data were conducted with PAUP* v. 4.0b10 for Windows (Swofford 2002). The most parsimonious trees from analyses applied a heuristic search using 1000 random addition sequences (RAS), TBR branch swapping algorithm, save multiple trees, collapse zero length branches when maximum length is zero, gaps treated as a fifth character state, characters given equal weight. A bootstrap analysis of 1000 replicates with five RAS per replicate, TBR branch swapping was then conducted. Additional support values for first and second data set were further estimated with maximum likelihood rapid bootstrapping (MLb), employing rapid bootstrap heuristics in RAxML v. 7.2.8 as described above (Stamatakis et al. 2008).

Bayesian posterior probabilities (PP) ≥ 95 %, and MPbs and ML bootstrapping (MLb) ≥ 70 % were considered to be significant.

RESULTS

The LSU phylogeny

The LSU alignment (the first data set) contained 92 sequences with 853 total and 269 conserved sites. A Bayesian analysis yielded the phylogeny presented in Fig. 2. *Cantharellus* s. lat. (clade A) is strongly supported on a long branch (PP=1.0; MPbs=100; MLb=100), and *Craterellus* is the sister-group of clade A (PP=1.0; MPbs=100; MLb=96). In clade A there are two distinct and strongly
Table 3. Morphological features of Afrocantharellus and Cantharellus.

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

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=98) 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. nov.**

MycoBank MB518687

*Basionym: Cantharellus* subgen. *Afrocantharellus* Eyssart. & Buyck, *Docums Mycol*. *Brux*. **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

2 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*

3 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

4 Stipe, cap margin, and folds with glaucous or bluish tinges ................................................. 3. *A. platyphyllus f. cyanescens*

4 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: 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, miombo dominated by Brachystegia, Combretum and Julbernardia, April 2007, 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. 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, distinctly 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 ellipsoidal 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).
Afrocantharellus gen. nov.

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 (Nyangbo dialect): Binyantuku. Burundi (Kirundi dialect): Peri magufa.

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. platyphylus 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.
**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 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. longisorus*, 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); 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. roridulus*, which was placed in subgen. *Rubinus* (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 (tet-
analyses that some species names used for sequences in Hofstetter 2011, Buyck included are A. platyphyllus all monophyletic groups within former that we do not have clear morphological synapomorphies for of genera with a rather uniform morphology where few should not discourage the recognition of further taxa in the Eyssartier & Buyck (2001) referred these connections.

lack clamp by having a well-differentiated hymenophore with diverging symoensii part above. It consists of species closely related to misleading in the study of these difficult taxa (Buyck & species to 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.

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