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Lactifluus kigomaensis and *L. subkigomaensis*: Two look-alikes in Tanzania

Ruben De Lange [a, *], Eske De Crop [a], Lynn Delgat [a], Donatha Tibuhwa [b], Deo Baribwegure [c], Annemieke Verbeken [a]

[a] Ghent University, Dpt. Biology, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium
[b] Dpt. Molecular Biology and Biotechnology, University of Dar Es Salaam, P.O. Box 35179, Dar Es Salaam, Tanzania
[c] KICORA, P.O. Box 909, Kigoma, Tanzania

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ABSTRACT

A look-alike of *Lactifluus kigomaensis*, described in 2012 from primary miombo woodlands in the Kigoma Province of northwestern Tanzania, is proposed here as *L. subkigomaensis*. The phylogeny based on the molecular markers ITS, LSU, *RPB1* and *RPB2* shows that *L. subkigomaensis* is a sister species to *L. kigomaensis*. Detailed descriptions of both species are given here, aiming at finding good characters to unravel these look-alikes. Both species are consumed and offered for sale on local markets.

1. Introduction

Miombo woodland is one of the most important and richest ectomycorrhizal vegetation types of Africa. In Tanzania, the Kigoma province is the region containing the largest untouched miombo zones in the country. Edible mushrooms within miombo woodland are well-explored in Tanzania, however, not in Kigoma (Härkönen, Saarimäki, & Mwasumbi, 1994, 1995, 1998, 2003, 1993; Saarimäki, Härkönen, & Mwasumbi, 1994; Calonge, Härkönen, Saarimäki, & Mwasumbi, 1997; Karhula, HÄrkönen, Saarimäki, Verbeken, & Mwasumbi, 1998; Tibuhwa, Buyck, Kivaisi, & Tibell, 2008, 2012; Buyck, Kauff, Couloux, & Hofstetter, 2012).

This paper reveals two species behind the formerly described *Lactifluus kigomaensis* De Crop & Verbeken. In its original publication, *L. kigomaensis* was mentioned to share quite some characters with species of *L.* sect. *Pseudogymnocarpi* (Verbeken) Verbeken (thick-walled hairs in the pileipellis and lamprocystidia), but because of the trichodermic structure of the pileipellis it was deviating from other representatives in this section. The preliminary phylogenetic results at that time suggested that *L. kigomaensis* had an isolated position within the phylogeny of *Lactifluus* (Pers.) Roussel. In the meantime new phylogenetic results show that *L. kigomaensis* is actually two species and is placed within *L.* sect. *Rubroviolascentini* (Singer) Verbeken, an exclusively African clade within *L.* subg. *Pseudogymnocarpi* (Verbeken) De Crop, of which all species are characterised by pleurolamprocystidia (De Crop et al., 2017). Other representatives of *L.* sect. *Rubroviolascentini* are L. *carmineus* (Verbeken & Walleyn) Verbeken, *L. rubroviolascens* (R. Heim) Verbeken and L. *denigricans* (Verbeken & Karhula) Verbeken.

Since the publication of *Lactifluus kigomaensis* in 2012 (De Crop, Tibuhwa, Baribwegure, & Verbeken, 2012), our knowledge on the diversity of *Lactarius* Pers. and *Lactifluus* in tropical Africa has increased fairly, bringing the total number to 41 (instead of 39) and 75 (instead of 59) species respectively (Maba, Guelly, Yorou, & Agerer, 2015a, 2014a, b, b; Beenken, Sainge, & Kocyan, 2016; De Crop, 2016; De Crop et al., 2016; Delgat, De Crop, Njouonkou, & Verbeken, 2017; Maba, 2015). But even more important, our insight in the genus *Lactifluus* has changed considerably and led to a new classification (De Crop, 2016; De Crop et al., 2017). In this new classification, *Lactifluus* is divided into four subgenera and 19 sections. *Lactifluus* subg. *Gymnocarpi* (R. Heim ex Verbeken) De Crop

* Corresponding author.
E-mail address: ruben.delange@ugent.be (R. De Lange).

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consists of 4 sections, *L.* subg. *Lactariopsis* (Henn.) Verbeken consists of 4 sections, *L.* subg. *Lactifluus* consists of 6 sections and *L.* subg. *Pseudogymnocarpi* consists of 5 sections. All subgenera, except *L.* subg. *Lactifluus*, contain even more unknown clades that probably represent new sections (De Crop, 2016; De Crop et al., 2017).

In De Crop et al. (2012), collection AV 11-006 was chosen as holotype of *L. kigomaensis* but the microscopic description and line drawings were based on collection AV 11-066. Molecular analysis revealed that collections AV 11-006 and AV 11-066 represent two different species. Consequently, the original description of *Lactifluus kigomaensis* is actually a mixture, with a macroscopic description based on the holotype collection and a microscopic description based on a macroscopically almost identical sister species of *L. kigomaensis.* This work rectifies the description of *Lactifluus kigomaensis* and describes a second new *Lactifluus* species from the Kigoma province in Tanzania, *Lactifluus subkigomaensis* De Lange & De Crop sp. nov. An additional analysis also showed that collection EDC 11-013 was wrongly mentioned in the studied material of De Crop et al. (2012). The collection does not represent *L. kigomaensis* nor *L. subkigomaensis,* nor a closely related species.

2. Materials and methods

2.1. Sampling

During field work in 2011 from March until April in miombo woodlands in the Kigoma province (Northwestern Tanzania), two collections of *Lactifluus kigomaensis* and four collections of *L. subkigomaensis* were found (from collection EDC 11-018 there is no dried specimen available, only a CTAB sample). The studied collections are deposited in herbarium Universitatis Gandavensis (GENT).

2.2. Morphological analysis

The macroscopic description was based on fresh material. Microscopic characters were studied from dried material in Congo-red in SDS (Sodium dodecyl sulfate). Spore ornamentation was observed in Melzer's reagent. For the terminology used we refer to Verbeken (1998) and Verbeken and Walleyn (2010). Line drawings were made with the aid of a drawing tube (Zeiss camera lucida on a Zeiss Axioskop 2 microscope equipped with a magnification changer of 2.5× for spores, without magnification changer for sections and an Olympus U-DA on an Olympus CX21 microscope for individual elements) at original magnifications of 6000× for spores and 1000× for sections and 1500× for individual elements. Pleurolamprocystidia were measured at original magnification of 400× . Basidia length excludes sterigmata length. Spores were measured in side view in Melzer's reagent, excluding the ornamentation. Spore measurements are given as (MINa) [AVa-2*SD]—AVa—AVb—[AVb+2*SD] (MAXb), with AVa = lowest mean value for the measured collections and AVb = greatest mean value for the measured collections, SD = standard deviation, MINa = lowest extreme value of collection “a” and MAXb = greatest extreme value of collection “b”. The Q-value (quotient length/width) is given as (MIN Qa) Qa—Qb (MAX Qb), with Qa = lowest mean ratio for the measured collections and Qb = greatest mean ratio for the measured collections, MIN Qa = lowest extreme ratio of collection “a” and MAX Qb = greatest extreme ratio of collection “b”. For the color codes, we refer to Kornerup and Wanscher (1978).

2.3. Molecular analysis

DNA from dry collections was extracted using the protocol described by Nuytinck and Verbeken (2003), with modifications described in Van de Putte, Nuytinck, Stubbe, Le, and Verbeken (2010). DNA from fresh material was extracted using the CTAB extraction described in Nuytinck and Verbeken (2003). Protocols for PCR amplification follow Le, Nuytinck, Verbeken, Lumyong, and Desjardin (2007). Four nuclear markers that were previously shown informative within this subgenus (De Crop et al., 2017) were used: (1) the internal transcribed spacer region of ribosomal DNA (ITS), comprising the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, using primers ITS-1F and ITS4 (Gardes & Bruns, 1993; White, Bruns, Lee, & Taylor, 1990), (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo, Lutzoni, Rehner, Johnson, & Vilgalys, 2000), (3) the region between the conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (*RPB2*), using primers bRPB2-6F and fRPB2-7cR (Liu, Whelen, & Benjamin, 1999; Matheny, 2005) and (4) the region between domains A and C of nuclear gene encoding the largest subunit of RNA polymerase II (*RPB1*), using primers RPB1-Ac and RPB1-Cr (Matheny, Liu, Ammirati, & Hall, 2002; Stiller & Hall, 1997). When necessary, internal primers RPB1- F3 and RPB1-R4 were used (De Crop et al., 2017). PCR products were sequenced using an automated ABI 3730 XL capillary sequencer at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with the SequencherTM v5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA).

We know from De Crop et al. (2017) that *Lactifluus kigomaensis* and *L. subkigomaensis* belong to *L.* subg. *Pseudogymnocarpi*, *L.* sect. *Rubroviolascentini*. Our dataset contains the *L.* subg. *Pseudogymnocarpi* sequences from De Crop et al. (2017), including all known representatives *of L.* sect. *Rubroviolascentini*. One sequence per species was used, except for *L. kigomaensis* and *L. subkigomaensis,* of which more sequences were added. Five species of *L.* subg. *Lactifluus* were used as outgroup (Table 1).

Sequences were aligned using the online version of the multiple sequence alignment program MAFFT v7 (Katoh & Toh, 2008), using the E-INS-I strategy. Trailing ends of the alignments were trimmed and the alignments were manually edited when necessary in Mega 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). The alignments can be obtained from the first author and TreeBASE (Submission ID 20557). The alignments were partitioned into following partitions: ITS-LSU-alignment: partial 18S, ITS1, 5.8S, ITS2, LSU; *RPB2*-alignment: the *RPB*2 intron and the first, second and third codon positions of the exon, and *RPB1*-alignment: the different *RPB*1 introns and the first, second and third codon positions of the exons. Maximum likelihood (ML) analyses were conducted with RAxML v8.2.10 (Stamatakis, 2014), where a ML analysis was combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis, Hoover, & Rougemont, 2008). Analyses were performed on each alignment separately. The resulting gene trees did not show any supported conflicts, therefore all gene trees could be concatenated. The concatenated tree was used in the Results. All analyses were performed on the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010).

3. Results

Our molecular results clearly show that collections AV 11-006 and AV 11-066 represent two different species (Fig. 1). The new species is a sister species of *Lactifluus kigomaensis* (Fig. 1). This is supported by morphological differences (see Discussion). Based on these morphological and molecular differences, the new species is here described as *Lactifluus subkigomaensis* sp. nov. A revised description of *L. kigomaensis* is provided (see Fig. 2).

Lactifluus kigomaensis De Crop & Verbeken, *Cryptogamie*

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