Multigene sequencing provides a suitable epitype, barcode sequences and a precise systematic position for the enigmatic, African Cantharellus miniatescens

Bart BUYCK\textsuperscript{a*}, Terry W. HENKEL\textsuperscript{b}, Bryn T. M. DENTINGER\textsuperscript{c}, Olivier SÉNÉ\textsuperscript{d} & Valérie HOFSTETTER\textsuperscript{e}

\textsuperscript{a}Muséum national d’Histoire naturelle, département systématique et Évolution, CP 39, ISYEB, UMR 7205 CNRS MNHN UPMC ÉPHE, 12 rue Buffon, F-75005 Paris, France

\textsuperscript{b}Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, USA

\textsuperscript{c}Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

\textsuperscript{d}Institute of Agricultural Research for Development, National Herbarium of Cameroon, PO Box 1601, Yaoundé, Cameroon

\textsuperscript{e}Department of plant protection, Agroscope Changins-Wädenswil research station, ACW, rte de duiller, 1260, Nyon, Switzerland

\textbf{Abstract} – Cantharellus miniatescens is lectotypified. An epitype sequenced for four gene regions (LSU, mitSSU, RPB2 and Tef1-alpha) is selected among recent collections from Cameroon and Central African Republic and fully described and illustrated. Complete ITS sequences have been deposited as barcodes. The systematic position is determined using a multigene phylogenetic analysis which places this species in Cantharellus subg. Pseudocantharellus in agreement with its morphological features.

Barcoding / Epitypification / Lectotypification / phylogeny / taxonomy

\textbf{INTRODUCTION}

The diversity of Cantharellus appears to be much higher in tropical Africa and Madagascar than on any other continent and new species continue to be described in rapid succession (Ariyawansa \textit{et al.}, 2015; Buyck 2012a, 2012b; 2014; Buyck \textit{et al.} 2000, 2013, 2014, 2015, 2016a,b; De Kesel \textit{et al.}, 2011, 2016). In several of these recent papers, the first author has already exposed the problematic identification of tropical chanterelles resulting from the uncertainty that surrounds most of the species concepts of the nearly 30 Central African chanterelles described more than half a century ago (Heinemann 1958, 1966). This applies especially to the rain forest

\* Corresponding author: buyck@mnhn.fr
taxa most of which have never been recollected since their original description. These rain forest chanterelles urgently need more precise and fully illustrated descriptions and, most importantly, multigene DNA sequence data allowing for more reliable identification through epitypification. Indeed, the type specimens for most of these species have been collected almost one century ago and annihilate chances for successful extraction of good quality DNA for sequencing purposes (see De Kesel et al. 2016, this issue). Whereas epitypification recently started for several problematic chanterelles from the African woodlands and Madagascar (Buyck 2012a, Buyck et al. 2016b), this paper proposes the lecto- and epitypification of C. miniatescens Heinemann (1958) on the basis of recently collected specimens from the tropical African Gilbertiodendron dewevrei rain forest, its original habitat.

_Cantharellus miniatescens_ was described by Heinemann (l.c.) on the basis of dried specimens accompanied by some notes and watercolors provided by Mrs Martha Fontana who was collecting and illustrating fungi from the African rain forest during the first half of the 20th century when she was accompanying her husband, V. Goossens, to the then “Belgian Congo” where he was appointed director of the Botanic Garden of Eala, before moving to Binga, near Lisala, in charge of various agricultural projects (Robyns, 1968).

The holotype consists of six minute specimens in bad condition, some collected in 1942 and others in 1946, most probably not from exactly the same locality. The heterogeneous holotype seems incompatible with the original description because of the extremely small size (< 12 mm) of the specimens, the non-intervened, very dense and narrow gill-folds, as well as some deviating microscopical features (Eyssartier 2001). Furthermore, the paratype (de Loose B35) is an unrelated species lacking clamp connections (Eyssartier, I.c.) that was collected from woodland vegetation. No other original material of this chanterelle exists neither at BR nor elsewhere. Subsequently, the name _C. miniatescens_ was considered a _nomen dubium_ (Eyssartier, I.c.) that was impossible to interpret without recollecting new material.

Notwithstanding the uncertain and vague species concept of _C. miniatescens_, this chanterelle has been mentioned in several publications that discuss common edible mushrooms in Africa (e.g. Eyi Ndong et al. 2011; Malaisse 1997, 2010; Boa 2004) and was even reported from other continents (Kumari et al. 2013, for a collection made under _Cedrus_ in the Indian Himalayas). It is furthermore the sole African chanterelle repeatedly illustrated on post stamps from several African countries (Zaire 1979 [clearly based on the original description but too brownish], Zambia 1984 and Zimbabwe 2006 [both in reality _C. symoensii_]). The most recent illustrated report of _C. miniatescens_ (Eyi Ndong et al. 2011, fig. 79) deviates clearly from the original description by the very well-developed, non-recurrent and non-forking, too widely spaced (5/cm instead of 10-12/cm) and very large gill folds without intervention. The latter concept can be attributed to some of the inconsistencies mentioned in the original description, and corresponds in reality to a common _Hygrocybe_ from the Central African rain forest as suggested by sequences obtained from morphologically identical specimens from Cameroon (Buyck, not shown). Indeed, Heinemann (1958) mentioned for _C. miniatescens_ an overall resemblance to _Hygrocybe_, a comparison which is difficult to understand given the strongly forked, deeply decurrent, very low, vein-like and reticulately anastomosing gill-folds illustrated by Goossens-Fontana for this chanterelle. Heinemann also wrote “_lamellae distantes_” in the latin diagnosis, which is absolutely not the same as the mention of “gills not particularly close (L + 1:10-12/cm)” in his more detailed French description. Also the gill folds were described by Heinemann as “roseo-croceae” in the latin diagnosis, while the more precise French description reads “with yellow
and pinkish tones, sometimes white”. The pinkish color certainly applies to the gills of the *Hygrocybe*, but not to those of *C. miniacescens* which were left white in the original watercolor (see fig. 5).

In this contribution, we present several collections from Cameroon and from neighboring Central African Republic that correspond perfectly to Goossens-Fontana’s original watercolors and Heinemann’s detailed description of this enigmatic species. These specimens were collected in the original habitat, i.e. *Gilbertiodendron dewevrei*-dominated rain forest, with one of the locations being merely some 350 km away from the holotype locality. The species is here lecto- and epitypified, barcoded, fully redescribed and illustrated and its systematic position determined on the basis of a multigene phylogeny.

**MATERIAL AND METHODS**

*Collecting.* In Cameroon, basidiomata were collected during the Aug.-Sept. early rainy season of 2014 from the Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within a two km radius of a base camp located at 3°21’29.8” N; 12°43’46.9” W, 650 m a.s.l., in forests dominated by *G. dewevrei* (Peh et al. 2014). The Central African Republic collections were made this year during early May in pure *Gilbertiodendron dewevrei* patches in Dzangha Sangha Forest Reserve notwithstanding very dry weather conditions. Photographs and descriptions of macromorphological features were made from fresh material in the field. Colours were compared with colour plates from Kornerup & Wanscher (1978) and are cited in parentheses. Collections were dried with silica gel (Cameroon) or in a self-made drier (RCA). Epitype material and additional specimens are deposited in PC, Muséum national d’histoire naturelle, Paris, and for the Cameroon collections also in the following herbaria: YA, Cameroon National Herbarium; HSC, Humboldt State University; K(M), Fungarium, Royal Botanic Gardens, Kew.

*Microscopic study.* All sequenced specimens were microscopically examined by the first author in ammoniacal Congo red after a short pre-heating in KOH, at a magnification of ×1,000. Drawings and measurements of individual elements were made for every specimen at a drawing magnification of ×2,400 using a camera lucida and follow protocols as described in Buyck et al. (2014).

*Molecular study.* Extraction, amplification and sequencing of five gene regions (LSU, RPB2, tef-1, mitSSU and ITS) follow protocols described in Buyck et al. (2014). Sampling and phylogenetic analyses for this study are identical to Buyck et al. (2016a, this issue).

### Table 1. Genbank submissions for newly produced sequences used in the multigene analyses

<table>
<thead>
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<th>Voucher</th>
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<td>mitSSU</td>
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<td>1682/TH9852 epitypus</td>
<td>Cameroon</td>
<td>PC 142437</td>
<td>KX857133</td>
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<td>1683/TH9870</td>
<td>Cameroon</td>
<td>PC 0142438</td>
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Fig. 1. Part of the most likely tree depicted in Buyck et al. (2016a this issue, see Fig. 1) showing the position of *C. miniatesscens* as part of *Cantharellus* subg. *Pseudocantharellus.*
RESULTS

Molecular results

Phylogenetic analyses (Fig. 1) place C. miniatescens (ML-bs 100%) in a monophyletic clade with C. subincarnatus ssp. rubrosalmonéus with significant support (ML-bs = 77%). This clade corresponds to clade 5 (Cantharellus subg. Pseudocantharellus) in Buyck et al. (2014). Specimen 1683 (TH9870) from Cameroon appears to be distinct from the two other collections (monophyly of 1671 and 1682: ML-bs = 100%) as the result of a few mutations in the LSU and 3rd codon positions in the RPB2. This specimen interestingly corresponds to the collection with a less developed hymenophore (see Figs 8, 11) but more collections are needed to decide whether it deserves recognition as a separate variety or form of this species.

We also produced barcode ITS sequence data for this species. We failed to obtain ITS sequences for the Cameroon material (thus including the holotype), but succeeded to obtain identical and complete ITS sequences (1135 base pairs) for the two collections from Central African Republic; this sequence has been deposited as representative sequence for this species on GenBank (nr. KX857082).

Taxonomic results


Figs 2-12


Original description (freely translated from French): “Cap 3-6 cm diam., thin-fleshed, convex and with an umbilicate cap when young, becoming broadly campanulate with a strongly depressed center surrounded by coarse, radial sulcations; margin first inrolled, then more straight to even uplifted, lobed to crenulate; the surface tomentose, orange-yellow with reddish tones, particularly where protected from light. Stipe 3-4 cm x 3-9 mm, cylindrical or narrowing downward, rarely more inflated near the base, fibrillose to silky, solid or sometimes becoming fistulose, white with orange and pinkish tones, becoming distinctly yellowish with age. Gills not particularly close (L + l: 10-12/cm), plicate, narrow, up to 2 mm high, deeply decurrent, with yellow and pinkish tones, sometimes white, anastomosed-forked with many irregular, more or less transversal veins. Flesh firm, white, yellowish or orange beneath the surface; odor strong and typical (of C. cibario) still noticeable after boiling up specimens several decennia after drying; taste acrid, then bitterish. Spore print white. Exsiccateum entirely orange brown.

Spores hyaline or faintly yellowish, 7-8,3 × 4,3-5,1 μm, ellipsoid, sometimes slightly depressed on the inner side, outer episporo possibly finely rugose; perispore yellowish. Basidia slender, 39-50 × 8-10,5 μm, (4-)6-spored. Pseudoparenchyma well-differentiated in the cortical part of the stipe. Cap cuticle not squamulose, composed of more or less radially oriented, thin-walled hyphae, 5-11 μm diam., clamped, when fresh containing yellow to pinkish refringent guttules, with free, narrowly clavate to lanceolate endings; with dispersed oleiferous hyphae with clavate extremities.

CENTRAL AFRICA. Democratic Republic of Congo (previously Belgian Congo, then Zaïre): Central district, Binga, Bonsendo-Mongana, on the soil of the Gilbertiodendron dewevrei forest, often covered by fallen leaves, July 1942 and 1946, M. Goossens-Fontana 2086 (holotype BR); District du Haut-Katanga, Elisabethville, January 1933, de Loose B35 (paratype BR).”
Fig. 5. C. miniatescens, lectotypus (reproduced from Flore Iconographique des Champignons du Congo, pl. XXVI, fig. 1. 1959; with permission National Bot. Garden Belgium).

Lectotypification: because the specimen that is kept at BR as holotype for this species does not (or no longer) correspond to the specimen illustrated in the original watercolor (see our Fig. 5) by Mrs. Goossens-Fontana, we choose the following illustration here as lectotype for C. miniatescens:

Iconography: HEINEMANN 1959, Flore Iconographique des Champignons du Congo, pl. XXVI, fig. 1, lectotypus hic designatus.

Epitypification:
Basidiomata growing in large troops, composed of up to several hundreds of specimens dispersed or in subcaespitose clusters, among the fallen leaves on the forest floor. Pileus 11-95 mm diam. and very thin-fleshed, broadly convex in the
earliest stages with strongly inrolled margin, then rapidly becoming depressed in the center to distinctly umbilicate with the marginal zone remaining downturned, finally sometimes infundibuliform often with locally lobed, wavy or uplifted margin; cap surface outside the center at maturity often broadly and radially sulcate following the pattern of the principal gill-folds underneath, when very young entirely pinkish-vinaceous to purplish red (10-11D7) and covered with sub-erect squamulae, retaining this squamulose aspect in the center when expanding, becoming densely matted to subglabrous elsewhere; light orange (5A7) or paler when dry or old, especially when young with pinkish-vinaceous tints (6-7A4) which persist the longest in the center and near the extreme cap margin, elsewhere usually fading to yellowish. **Hymenophore** strongly decurrent over one third of the stipe, sharply delimited from the rest of the stipe surface, composed of forking, blunt and low gill-folds up to 4 mm high and strongly interveined and anastomosing in between, rarely also with very low veins, ca 1 mm high and without interveination in between the radial veins, white, then with pale yellowish to pale orange (5A3-4) tones, occasionally with pinkish shades (6A2-3), remaining often pure white on the gill edges and close to the stipe surface. **Stipe** central, 23-82 × 3-9 mm, where covered by the hymenophore widening to sometimes 15 mm diam., not rooting, glabrous to minutely fibrillose or striate under a hand lens, pale yellow to orange (5A3-5), sometimes locally pinkish, often developing a chalk white zone at the basal and apical part, subsolid, fibrous, off-white inside. **Flesh** very thin (< 1 mm thick) outside the cap center, off-white, unchanging. **Taste** typical but weakly of chanterelles (apricot), pleasant. **Odor** typical, fruity. **Spore print** not obtained. **Exsiccatum** with greyish brown cap surface lacking any pinkish or yellowish-orange colors.

*Fig. 6. C. miniatescens*, epitypus, field habit (TH 9852). Photo Todd Elliott.*
Fig. 11. *C. miniatescens*, field habit (TH 9870). Photo Todd Elliott.

Fig. 12. *C. miniatescens*, field habit (BB16.030). Photo B. Buyck.
**Basidiospores** ellipsoid to narrowly ellipsoid, frequently slightly constricted in the middle or peanut-like to reniform, (6.9)7.0-7.45-7.9(8.3) × (3.7)3.8-4.18-4.5 (5.2) μm, Q = (1.5)1.6-1.79-1.9(2.1), smooth. **Basidia** medium-sized, 45-55(-60) × 7-8 μm, subcylindrical to (very) narrowly clavulate, not strongly undulate as typical for many other species, (4-)5(-6)-spored, basidia slightly more undulating and more clavulate just before sporulation. **Cystidia** not observed. **Subhymenium** filamentous, not strongly developed and composed of rather short cylindrical cells. **Pileipellis** composed of thin-walled, subcylindrical hyphae, 4-8(-11) μm diam., mostly running parallel, sometimes in fascicles on the surface, in deeper layers with some dispersed narrow oleiferous fragments; some hyphae with zebroid incrustations on their entire wall surface. Terminal cells morphologically hardly differentiated but with distinct refringent-granular contents of pigments, cylindrical, obtuse, of similar diam., mostly (20-)35-60(-110) μm long. **Clamp connections** very abundant in all tissues.

**Cameroon. East Province:** Dja Biosphere Reserve, Northwest Sector near the village of Somalomo, Upper Dja River Basin, within 2 km radius of Dja base camp located at 3°21’29.8” N; 12°43’46.9” W, 1 km west of Dja base camp, in monospecific upper story *Gilbertiodendron dewevrei* forest, on soil amidst thick litter layer, 650 m alt, coll. T. Henkel, M. Chin, T. Elliott, C. Truong, O. Sene, Ebambe, 15 August 2014, TH 9852 (YA, *epitopy hic designatus*, duplicates at HSC, PC 0142437 and K(M)).

**Additional collections studied:** CAMEROON. Dja Biosphere Reserve, 1.5 km West-North West from Dja base Camp, in monospecific upper story *Gilbertiodendron dewevrei* forest, on soil amidst thick litter layer, ca 650 m alt, legunt T. Henkel, M. Chin, T. Elliott, C. Truong, O. Sene, Ebambe, 20 August 2014, TH 9870 (YA; PC 0142438; HSC; K(M)).

**Central African Republic.** Dzanga-Sangha Forest Reserve, near Bayanga, close to Bai-Hakou base camp located at N 02.859934-E 16.467492, in monospecific upper story *Gilbertiodendron dewevrei* forest, on sandy soil amidst thick litter layer, 16 May 2016, Buyck 16.030 (PC 0142439); ibid., 24 May 2016, Buyck 16.112 (PC 0142440).

**Discussion**

Our collections depict *Cantharellus miniatescens* as a small to medium-sized, rather slender and thin-fleshed species that starts out with an entirely vinaceous to reddish pileus resulting from a dense tomentose-hairy cover of minute fibrils (Fig. 9). At the onset of the cap expansion this surface layer is rapidly disrupted, thereby exposing the bright yellow to orange yellow surface underneath which rapidly determines the general aspect of mature fruiting bodies (see for ex. Fig. 12). However, remnants of these fibrils remain mostly present at the extreme cap margin and also in the cap center and represent an easy means of identification. The hymenophore is definitely off-white to even chalk white and nearly always strongly veined – anastomosed in between the larger gill folds. The pinkish to yellowish tinges that were mentioned for the hymenophore in the original diagnosis and that were responsible for later misinterpretations of this species, are due to the transparency
of the surface color of the very thin-fleshed pileus. The mention of the taste being “acid, then bitterish” in the original notes of Mrs. Goossens-Fontana may be a consequence of her prolonged use of kinine (Heinemann, pers. comm.).

Following the multigene phylogeny of *Cantharellus* (Buyck et al. 2014) and recent emendation (De Kesel et al. 2016 this issue), the presence of abundant clamp connections in *C. miniatescens* would already exclude a placement in sections *Isabellinus* Eyssart. & Buyck and *Heinemannianus* Eyssart. & Buyck of the species-rich subg. *Rubrinus* Eyssart. & Buyck, a subgenus endemic to Africa and harboring the bulk of the African chanterelle species. For the same reason, it would also exclude a placement in the less diverse subg. *Afrocantharellus* Eyssart. & Buyck which groups several tropical African and Asian chanterelles with well-developed gill folds. The irregularly clamped chanterelles of the newly described sect. *Stramineus* in subg. *Rubrinus* (see De Kesel et al., 2016 this issue) all differ from *C. miniatescens* in their more robust, fleshy fruiting bodies with frequently squamulose surface. Among the four remaining subgenera, all having species with abundant clamps, subg. *Cantharellus* appears restricted to the northern hemisphere, while subg. *Parvocantharellus* Eyssart. & Buyck harbours nearly exclusively yellowish-brown species. This leaves either subg. *Cinnabarinus* Buyck & V. Hofstetter or subg. *Pseudocantharellus* Eyssartier & Buyck as most likely placement, both containing predominantly reddish species and both being either predominantly or exclusively reported from Africa.

Our phylogeny (Fig. 1) now indeed places this chanterelle with high support as sister to another African rain forest species, *C. subincarnatus* Eyssartier & Buyck, until now the only fully sequenced, other member of subg. *Pseudocantharellus*. The circumscription of the latter subgenus requires more sequence data on the type species, *C. ruber* Heinem., for which there are only partial ITS and partial LSU data available on GenBank. However, a preliminary analysis of our ITS sequence (not shown) places *C. miniatescens* in a monophyletic clade together with both *C. subincarnatus* and *C. ruber*, and thus seems to validate the circumscription of subg. *Pseudocantharellus*. Morphologically, *C. miniatescens* conforms well to the definition of this subgenus which was characterized (Buyck et al. 2014) as being composed of reddish-orange-pinkish species possessing abundant clamp connections and thin-walled hyphal ends at the pileus surface.

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