



## ***Cantharellus* subg. *Pseudocantharellus* (Hydnaceae, Cantharellales) revisited: one epitypification, one new synonym and one new species**

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### **Abstract**

*Cantharellus rhodophyllus* is epitypified with a recent collection from the African rain forest and full barcode ITS sequences are provided. After a detailed comparison of the original descriptions and these new collections, *C. subincarnatus* – introduced as a new name for the invalid *C. incarnatus* – is considered a later synonym. Phylogenetic analysis using *tef-1* sequence data place *C. rhodophyllus* in *Cantharellus* subg. *Pseudocantharellus* and demonstrate that *C. subincarnatus* subsp. *rubrosalmonus* from Madagascar is an independent species that is more closely related to *C. miniatescens*.

**Key words** – 1 new species – Barcoding – *Cantharellus rhodophyllus* – *Cantharellus subincarnatus* – *Cantharellus rubrosalmonus* – ITS – *tef-1*

### **Introduction**

Mainland Africa surpasses by far all other continents with its 44 presently described endemic *Cantharellus* species (De Kesel et al. 2016, Buyck 2016, Buyck et al. 2017). Yet, a major priority, addressed in several recent papers (Buyck et al. 2015, Buyck et al. 2016a), remains the urgent epitypification and molecular characterization of the many African chanterelles described by Heinemann (1958, 1966) in view of stabilizing the earlier introduced names and corresponding species concepts. Newly sequenced specimens that were recently collected in the African rain forest, in the same habitat, and not far from where Mrs. Goossens-Fontana made the collections that were later studied by Heinemann (l.c.), are now finally available to be compared with the original material and descriptions. Thus far, recent epitypifications of Heinemann's chanterelles allowed for the redescription, correct interpretation and systematic placement of *C. miniatescens* Heinem. and *C. rufopunctatus*, two previously misinterpreted species (Buyck et al. 2016b, De Kesel et al. 2016), as well as for *C. alboroseus* (Buyck et al. 2016a) and some species in the *C. congolensis*-complex (Buyck et al. 2016d).

In the present contribution, the authors address the existing confusion surrounding species recognition in *Cantharellus* subg. *Pseudocantharellus* with the epitypification of *C. rhodophyllus* Heinem. This epitypification additionally results in the recognition of a new synonym and a new species.

## Material and Methods

*Morphology.* – Color notations follow Kornerup & Wanscher (1978). Microscopic features were examined and sketched with the aid of a camera lucida. Original drawings for all elements of the hymenium or pellis were made at a magnification of 2400×. All microscopic observations and measurements were made in ammoniacal Congo red after a short pretreatment in a 10% aqueous KOH solution to improve tissue dissociation and matrix dissolution. Measurements of basidiospores cite length, width and length/ width ratio (Q) in this format: (minimum–) mean minus standard deviation – mean value – mean plus standard deviation (– maximum measured); spore measurements are based on 20 spores.

*Taxon sampling and Phylogenetic analyses.* – Genomic DNA isolation, amplification and sequencing of the transcription elongation factor 1-alpha (*Tef-1*) for the here newly analyzed *Cantharellus* collections were performed as described in Buyck et al. (2014) including some *Tef-1* sequences from previous publications (Buyck et al. 2016b) and using *Craterellus tubaeformis* as the outgroup.

The *Tef-1* alignment included 92 specimens and was performed by eye in MacClade 4.05 (Maddison & Maddison 2002). The alignment used for phylogenetic analyses included 629 characters after exclusion of three spliceosomal introns (full alignment length: 864 bp). Searches for optimal tree and branch robustness were conducted with the program PhyML (Guindon & Gascuel 2003), with the search starting from a distance-based tree and with the proportion of invariable sites, the gamma shape parameter and the number of substitution categories estimated during the search. Three independent runs were conducted to check for convergence to the same likelihood value. Branch support was estimated based on 500 bootstrap replicates (ML-bs) and was considered significant when  $\geq 70\%$  (Alfaro et al. 2003)

## Results

### Phylogenetic results

The tree independent RAxML runs to search for the most likely tree exhibited nearly identical topologies and the one that maximizes the likelihood value ( $-\ln = 5713.37719$ ) is depicted in Fig.1. Phylogenetic analyses (Fig. 1) place *C. rhodophyllus* (ML-bs = 100%) monophyletic (ML-bs = 87 %) and sister (ML-bs = 77%) to a monophyletic group constituted by *C. miniatescens* and *C. rubrosalmoneus* sp. nov. (ML-bs = 100%). This clade corresponds to *Cantharellus* subg. *Pseudocantharellus* in Buyck et al. (2014). Identical barcode ITS sequences (1137 base pairs) were obtained for both our collections from the Central African Republic; these have been deposited in GenBank as barcode reference sequences (*C. rhodophyllus* 1638/BB16.006/PC 0142499, accession number MG450680; *C. rhodophyllus* 1678/BB 16.126/PC 0142500, accession number MG450681).

### Taxonomy

*Cantharellus rhodophyllus* Heinem., Bulletin du Jardin Botanique de l'État à Bruxelles 28: 404. 1958. Figs 2–4

= *Cantharellus subincarnatus* Eyssartier & Buyck, *Doc. Mycol.* 31 (121): 55 (2001), syn. nov

= *Cantharellus incarnatus* Heinemann, *Bull. jard. bot. État Brux.* 28: 402 (1958), *nom. inval.*

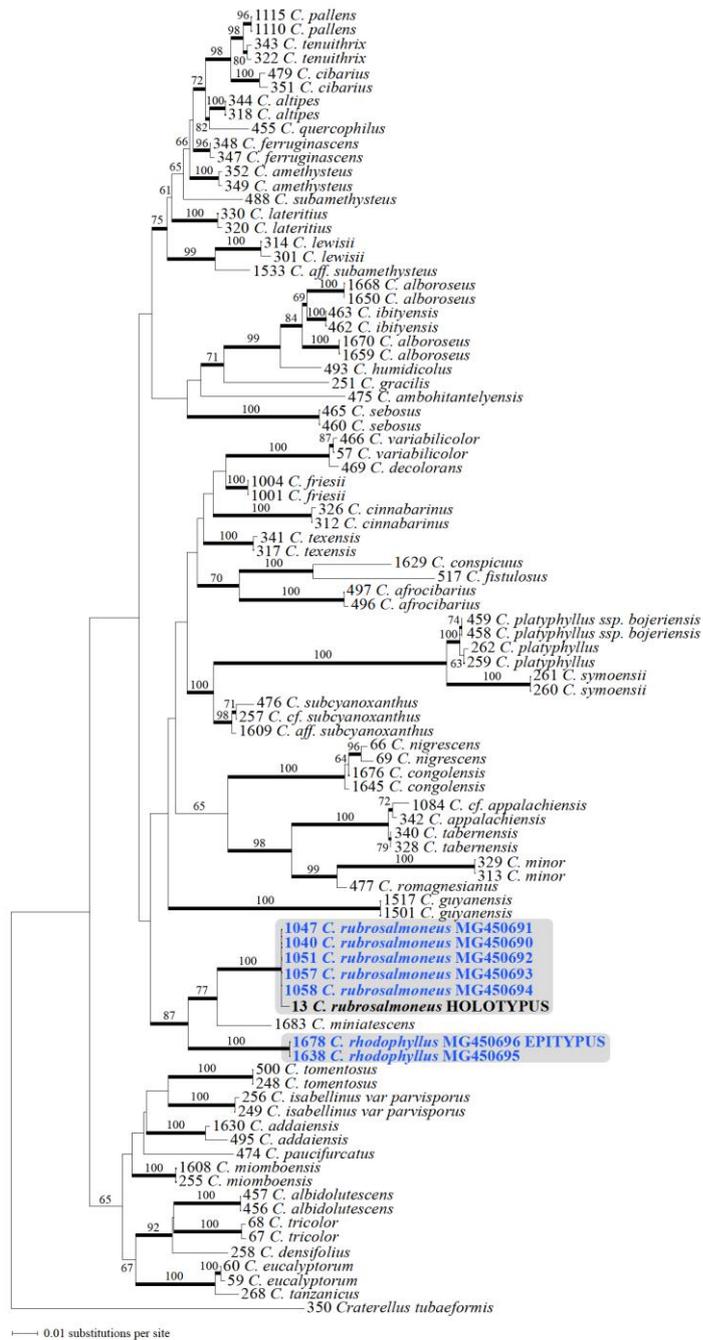
= *Lentinus incarnatus* Beeli, *Bull. Soc. Roy. Bot. Belg.* LX: 161, pl. IV, Fig. 24 (1928), basionym

Holotype: DEMOCRATIC REPUBLIC OF THE CONGO. Binga, on the soil in *Gilbertiodendron dewevrei* forest, July 1931, Miss Goossens-Fontana 937 (BR).

Iconography: Heinemann 1958, Fig. 41; 1959, pl. XXVII, Fig. 1a.

Diagnosis and original description (freely translated from French):

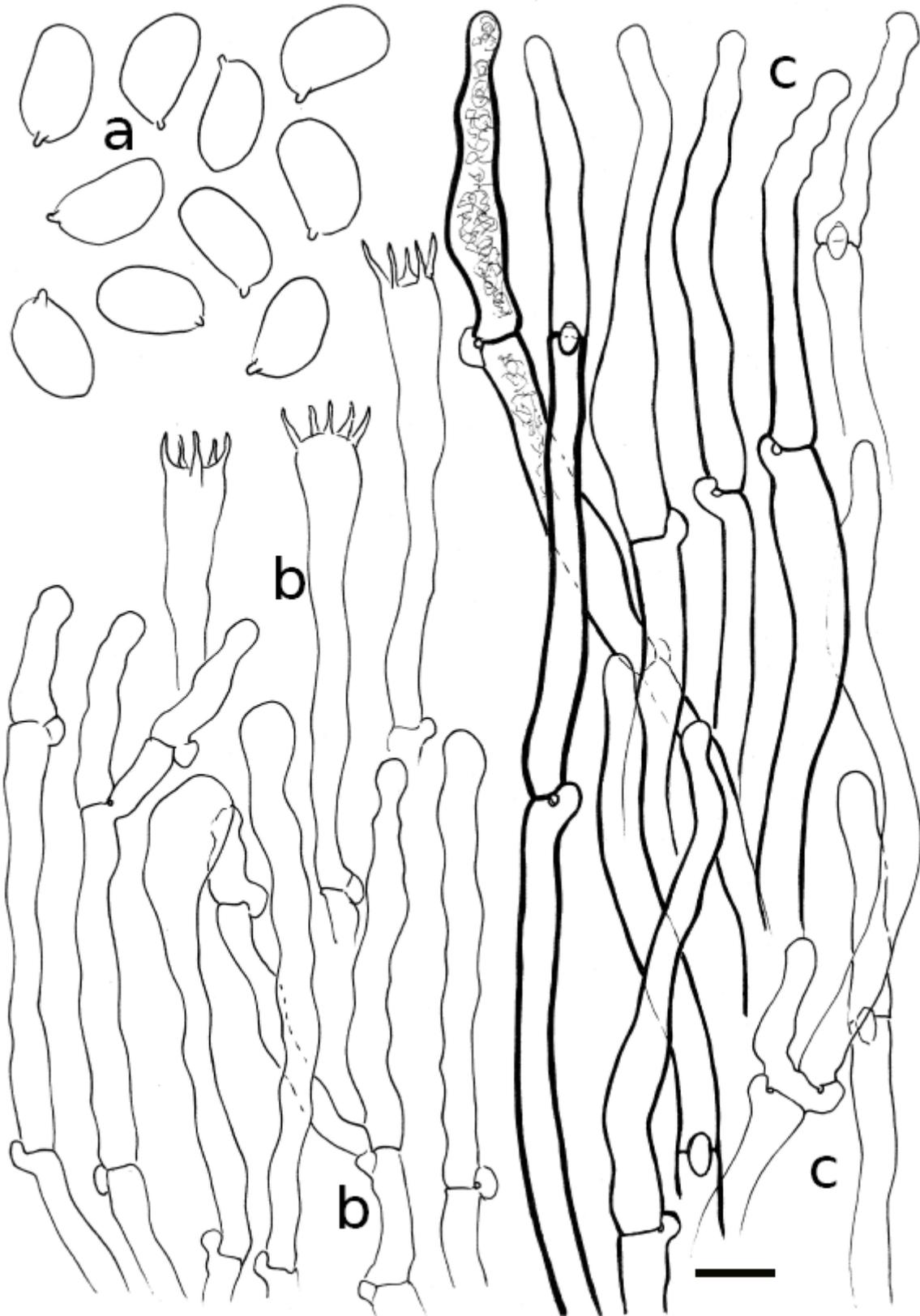
Pileus carnosus, centro depressus, lobatus, armeniacus, furfuraceo-hirsutus. Stipes validus, armeniacus-luteus, mycelio aureo praeditus. Lamellae angustae, confertiusculae, roseae, fragiles, furcatae et anastomosatae. Caro aquosa, crocea-luteola. Sporae 7-9 x 5,3-5,7  $\mu\text{m}$ , ellipticae.



**Figure 1** – Most likely tree obtained by analyses of 92 *Tef-1* sequences sampled from Buyck et al. (2016b). Branches significantly supported are in bold and bootstrap values are indicated along the branches. Newly produced sequences are highlighted in blue with taxon names followed by GenBank accession numbers.

*Pileus* ca. 12 cm diam., thick and fleshy, depressed in the center, with convex, irregularly lobed or undulating margin; surface a dark orange-pink, furfuraceous to even hirsute from small, fibrillose, vertically dressed and more or less detersile squamulae. *Stipe* ca. 13 x 2 cm, vigorous, irregularly cylindrical, tomentose (?), with shades of pinkish orange and bright yellow, not hollowing; mycelium bright ochre, visible at the base. *Lamellae* narrow, up to 4 mm high, rather densely spaced, thick and brittle, narrowing at the extremities, deeply decurrent, pinkish, unequal, forked, at their bases veined-anastomosing. *Context* watery, strongly fibrous, safran yellow especially in the stipe; when boiling a fragment of the exsiccatum releasing a weak odor of *C.*

*cibarius*. *Exsiccatum*: entirely reddish brown, basal mycelium bright red; with abundant, bluish to white punctate efflorescences on the entire surface.



**Figure 2** – *Cantharellus rhodophyllus* (epitype BB16.126). a Spores. b Basidia and basidiola. c Hyphal terminations in the pileipellis. Scale = 10  $\mu$ m, but only 5  $\mu$ m for spores.

Spores 7-9 x 5.3-5.7  $\mu\text{m}$ , ellipsoid, sometimes slightly depressed in the middle, thin-walled, with a small apiculus. *Basidia* 45-60 x 6.5  $\mu\text{m}$  for example (n°833 bis). *Pileipellis* with squamules formed of fasciculated yellowish hyphae with clamp connections, 4-10  $\mu\text{m}$  diam., thick-walled, with terminal elements narrowly lanceolate to cylindrical with obtuse tips. *Pseudoparenchyma* very compact, lacunar.



**Figure 3** – *Cantharellus rhodophyllus* (epitype BB16.126). Habit when fresh. Scale: one rectangle side on the paper equals 0.5 mm. Photo: B. Buyck.



**Figure 4** – *Cantharellus rhodophyllus* (epitype BB16.126). Detail of the scurfy-fibrillose cap surface. Photo: B. Buyck.

### Epitype description

*Basidiomata* dispersed, fleshy and firm, up to 70 mm high. *Pileus* entirely hairy-fibrillose and covered by distinct trichoid elements vertically dressed particularly toward the margin, brownish orange (7BC7-8, 7C6) in the center, deepening to brown or reddish brown elsewhere (7DE7-8, 8DE7). *Hymenophore* decurrent, composed of well-differentiated gill folds, up to 4 mm high,

unequal and near the cap margin spaced ca 1/mm, only rarely forking, not interveined, not abruptly delimited from the sterile stipe surface, very pale when young but then turning into a beautiful salmon color (6A5-7). *Stipe* subcylindrical, slightly narrowing downward, (25)40-45 x (6)10-22 mm, tinged with pink to salmon patches (7A5-77-8, 7B), massive and firm. *Context* firm and thick, sometimes appearing 'layered' in the upper stipe, strongly yellowing (also visible on the outer surface), particularly in the stipe lower half and there even turning ferruginous. *Smell* strong and typical. *Taste* mild. *Spore print* whitish.

*Spores* ellipsoid, (6.4)6.8-7.25-7.6(8.1) x (3.9)4.0-4.34-4.6(5.2)  $\mu\text{m}$ ,  $Q=(1.4)1.6-1.67-1.8$ , smooth, with small apiculus. *Basidia* 51-60(76) x 7-8  $\mu\text{m}$ , clavulate, predominantly 5-spored; sterigmata rather slender, 5-7  $\mu\text{m}$  long; basidiola long and slender, cylindrical, then finally apically swollen. *Subhymenium* filamentous, composed of long, slender, cylindrical cells. *Pileipellis* composed of long and slender, multi-celled hyphal extremities, adhering together in long tufts or trichoids, 4-6(8)  $\mu\text{m}$  diam., with relatively spaced septa and slightly thickened walls (only rarely up to 1  $\mu\text{m}$  thick), rapidly disintegrating in smaller units when making microscopic preparations; the terminal cell generally narrowing upward and often subcapitulate or repeatedly slightly constricted at the apex, (20)35-65(90)  $\mu\text{m}$  long, often with rather granular, not crystalline pigment inclusions. *Clamp connections* rather small and everywhere.

Examined and sequenced material: Central African Republic. Dzanga-Sangha Forest Reserve, near Bayanga, close to Bai-Hakou base camp, N 02.859934- E 16.467492, under monospecific *Gilbertiodendron dewevrei* forest, on bare sandy soil, 21 May 2016, Buyck 1638/BB 16.006 (PC 0142499); *ibid.*, 26 May 2017, Buyck 1678/BB 16.126 (PC 0142500, *epitypus hic designatus*).

Commentary: This species has been collected by Mrs Goossens-Fontana almost a century ago in the area of Binga, some 400 km across the border from where our specimens were gathered. Heinemann's description does not discuss the evident similarities shared between *C. rhodophyllus* and *C. subincarnatus* Eyssart. & Buyck (ut *C. incarnatus* Heinemann, *nom. inval.*), both of which are illustrated side by side (Heinemann 1959, Figs 1, 2). The Latin epithet of each species could equally apply to the other one as both were described with similar colors for cap, stipe and gills and both share a yellowing context. Moreover, both descriptions mention a cap surface covered with concolorous squamules and mention similar diameters for hyphal extremities on the cap surface; both descriptions also suggest a similar length-width ratio for spores although spore size is slightly different. Indeed, the spore size of *C. rhodophyllus* was described as 7 – 9 x 5.3 – 5.7  $\mu\text{m}$  versus 6.9 – 7.8 x 4.3 – 4.8  $\mu\text{m}$  for *C. subincarnatus* (Table 2). Notwithstanding the considerably wider spores of *C. rhodophyllus*, spores were illustrated (Heinemann 1958) exactly in the opposite way, viz. with the broadest spores for *C. subincarnatus*. The approximate length-width ratio (Q) for spores based on median values (in the absence of mean values) results in 1.45 versus 1.54 for *C. subincarnatus*, and this without accounting for Heinemann's mention of the occurrence of spores up to 11  $\mu\text{m}$  long in *C. subincarnatus* which would raise Q values >2). While Eyi Ndong et al. (2011) considered *C. rhodophyllus* as the species having the most elongate spores, the reexamination of both holotypes by Eyssartier (2001) suggested that Heinemann's measurements for *C. rhodophyllus* were most likely erroneous as Eyssartier measured near identical spore sizes for both holotypes and these correspond extremely well to spore measurements of our recently collected specimens (Table 1). Heinemann (1958) mentioned thick-walled hyphae in the pileipellis of *C. rhodophyllus* but he did not specify wall thickness for hyphal extremities in *C. subincarnatus* and illustrated both species as having thin-walled elements. Eyssartier (2001) noted for both type specimens similar, (very) faintly thickened cell walls (<0.5  $\mu\text{m}$ ). This corresponds quite well with our specimens and also agrees more with Heinemann's illustrations rather than with his descriptions. When considering the general field habit, clear distinctions between both descriptions are again difficult to find as the watercolors published by Heinemann (1959) depict for each species both a more slender, pale specimen as well as a more robust, darker specimen. Our specimens correspond very well to fig. 2b for *C. subincarnatus* in Heinemann (1959, ut *C. incarnatus*) and they clearly also resemble the specimens identified as such by Eyi Ndong et al. (2011), but they

share the yellowish stipe of the more robust *C. rhodophyllus* illustrated in Heinemann (1959: see his Fig. 1a).

In conclusion, we fail to find any significant differences between both type specimens and therefore conclude that they represent a single species as already suggested by both Eyssartier (2001) and De Kesel et al. (2016). The nomenclatural priority to name this species goes to *C. rhodophyllus*, thereby assigning the replacement name *C. subincarnatus* for *C. incarnatus* nom. inval. as a later synonym.

*Cantharellus rhodophyllus* may exhibit a context zonation similar as the one reported for *C. coccolobae* in subg. *Cinnabarinus* (see Buyck et al. 2016c). This occasional phenomenon concerns either whole fructifications or individual fruiting bodies and appears to have no particular taxonomic significance.

**Table 1** Comparison of obtained spore measurements for both species.

| <i>Cantharellus rhodophyllus</i>  |   |                                |
|-----------------------------------|---|--------------------------------|
| Heinemann (1958)                  | 7-9 x 5.3-5.7                                   | Not given                      |
| Eyi Ndong et al. (2011)           | (6.7)6.8-7.9-8.8 x (4.9)4.7-5.5-6.2(6.5)        | Q = (1.17)1.21-1.43-1.65(1.68) |
| Eyssartier (2001 holotype)        | 6.5-7.39-9 x 3.5-4.21-5                         | Q = 1.62-1.76-1.87             |
| 1678/BB16.126 = Epitypus          | (6.4)6.8-7.25-7.6(8.1) x (3.9)4.0-4.34-4.6(5.2) | Q=(1.4)1.6-1.67-1.8            |
| 1638/BB16.006                     | (6.4)6.7-7.10-7.5(7.9) x (3.9)4.0-4.28-4.5(4.8) | Q=(1.4)1.5-1.67-1.8(2.0)       |
| <i>Cantharellus subincarnatus</i> |   |                                |
| Heinemann (1958)                  | 6.9-7.8 x 4.3-4.8                               | Not given                      |
| Eyi Ndong et al. (2011)           | (6.9)7.0-7.7-8.4(8.8) x (4.1)4.2-4.6-5.1(5.3)   | Q= 1.47-1.66-1.85(1.90)        |
| Eyssartier (2001 holotype)        | 6-7.5-9 x 4-4.5-5                               | Q = 1.4-1.66-2                 |

***Cantharellus rubrosalmoneus*** (Buyck & V. Hofst.) Buyck & V. Hofst., comb. et stat. nov.

*Basionym:* *Cantharellus subincarnatus* subsp. *rubrosalmoneus* Buyck & V. Hofst., *Fungal Diversity* 70:203 (2015)

Additional examined and sequenced material: MADAGASCAR. East coast, Tamatave prov., at Ambila Lemaitso, in sandy soil under *Asteropeia multiflora*, *Sarcolaena* spp., *Leptolaena* spp., *Uapaca littoralis*, S18.87713-E049.13429, 27 June 2011, 1040/Buyck 11.038 (PC 0085571), *ibid.*, 1047/Buyck 11.052 (PC 0085578), Tampolo forest station, in sandy soil of littoral forest with *Uapaca* and *Sarcolaena* spp., S17.28719-E049.41478, 3 July 2011, 1051/Buyck 11.087 (PC 0085582), *ibid.*, S17.28454-E049.42875, 6 July 2011, 1057/ Buyck 11.133 (PC 0085588), *ibid.*, S17.28719-E049.41478, 8 July 2011, 1058/ Buyck 11.166 (PC 0713950).

Commentary: More recently, Buyck et al. (2015) described from Madagascar's east coast also a *C. subincarnatus* subsp. *rubrosalmoneus* Buyck & V. Hofst., which clearly constitutes an independent though closely related species in our phylogenetic analyses (Fig. 1) and is sister with significant support to *C. miniatescens* (ML bs = 77%). Consequently, we raise it here to species level.

This Malagasy species is easily distinguished from *C. rhodophyllus* because of its more reddish orange color, different cap surface texture and the much denser, less well-developed and often strongly anastomosing gill folds. It resembles more the probably closely related *C. ruber* Heinem. described from African woodland (Heinemann 1966).

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