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Almost one century later... *Cantharellus avellaneus* finally rediscovered!

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Abstract – The authors report on a brown chanterelle collected in the sandy soils of Madagascar's east coast. As this specimen agrees entirely with the protologue of *C. avellaneus*, it is here described and proposed as epitype for this apparently rare chanterelle that was first described in 1924 by Narcisse Patouillard. Its systematic placement as part of *Cantharellus* subg. *Parvocantharellus* sect. *Congolenses* is demonstrated using a multigene phylogeny and its complete ITS barcode sequence is provided.

Barcode / *Cantharellus congolensis* / *C. nigrescens* / epitypification / Madagascar / multigene phylogeny / systematics

INTRODUCTION

When starting a fungal inventory in any tropical country, one almost automatically assumes that the earliest species discovered and described from the area many decennia ago correspond logically also to the most common and easy-to-find species. This paper provides a convincing example of the opposite scenario.

Patouillard (1924) described only two chanterelles from Madagascar, *Cantharellus madagascariensis* and *C. avellaneus*, both from the same locality, Maromandia, in the Northeastern part of Madagascar (Prov. Mahajunga). Both specimens were collected and sent to him by Raymond Decary, a French administrator with a profound interest in nature working in Madagascar for nearly 27 years (Balard & Maestri, 2001). Notwithstanding the fact that the fungal inventory of Madagascar lists now an amazing 22 different species of *Cantharellus* (Buyck *et al.* 2015; Ariyawansa *et al.* 2015), both of Patouillard's chanterelles, the first to be described

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from Madagascar, remained an unsolved enigma to the present day. The essential features cited for *C. avellaneus* in the original description comprise a brown cap, dark-coloured gill folds that become mouse grey upon drying and rather elongate spores. Such dark-coloured gill folds represent quite an exceptional feature within the genus as they were essentially known from a single other species, *C. congolensis*. The latter species is a strongly blackening chanterelle described only a few years later (Beeli, 1928) from Central Africa as a species with rather small, elliptic spores and thin-walled hyphae with abundant clamp connections (Heinemann, 1958). The type specimens of both species were re-examined by Eyssartier (2001) who noted the strong overall resemblance of the microscopical features of both species, but he also confirmed the distinctly more elongate spores cited by Patouillard for *C. avellaneus* (7.5-*8.63*-10 × 3.5-*3.95*-4.5 µm. Q = 1.78-*2.19*-2.43) in comparison to spores of the *C. congolensis* type (Q = 1.33-*1.56*-1.78).

As nearly 20 years of fungal inventory missions to Madagascar by the first author and collaborators did not uncover any chanterelle that exactly corresponded to Patouillard's original description, the name "*C. avellaneus"* was finally applied, thereby explicitly ignoring that its spores were not conform to the original description, to one of the most common ectomycorrhizal associates of introduced eucalypts in Madagascar (Buyck 2008), a perfect look-alike of the Central African *C. congolensis*. In more recent years, however, Buyck (in Buyck *et al.* 2015) changed his opinion because all specimens of *C. congolensis* he had examined so far possessed very similar spores being constantly shorter compared to those of the type collection of *C. avellaneus*. As a result, the *congolensis* look-alike associated with eucalypts in Madagascar was described as a new close relative to the mainland *C. congolensis*, i.e. *C. nigrescens* Buyck & V. Hofstetter (in Ariyawansa *et al.* 2015) and Patouillard's species was considered to represent a still unknown, possibly related, but perhaps not blackening species that had never been recollected since it original description.

In this paper, we report on a brown chanterelle recently collected by one of us (E. Randrianjohany) in the sandy soils of Madagascar's east coast. As this specimen agrees entirely with the protologue of *C. avellaneus*, including identical spores, it is here described and proposed as epitype for this apparently very rare chanterelle.

MATERIAL AND METHODS

Morphology. – Color notations follow Kornerup and 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.

Molecular analyses. – For phylogenetic purposes we produced sequence data for the four genes (mitSSU, nucLSU, *RPB*2 and *TEF*-1) used in the *Cantharellus* phylogeny by Buyck *et al.* (2014) for two fruiting bodies from the epitype collection

of *C. avellaneus*. Following the barcode objectives outlined by Schoch *et al.* (2014), we also produced sequence data for ITS locus. Fungal genomic DNA isolation, amplification and sequencing were performed as in Buyck et al. (2014). Phylogenetic analyses were performed as described in Buyck et al. (2016, this issue).

RESULTS

Molecular results

We successfully obtained identical and complete ITS sequences (1135 base pairs) for each of the two fruiting bodies; the ITS barcode sequence has been deposited on GenBank. We added the other eight newly produced sequences for *C. avellaneus* (Table 1) to the alignment used in Buyck *et al.* (2016, this issue). The final alignment used for phylogenetic inference included 4 loci, 96 taxa and 3330 characters.

Table 1. Genbank accessions for newly produced sequences used in this study

Voucher	*Provenance*	*Accession PC*	*Genbank accession numbers*				
			mitSSU	*nucLSU*	*RPB2*	*TEF-1*	*ITS*
1217/ER	Madagascar	PC 0713845	KX857119	KX857093	KX856997	–	KX857081

Phylogenetic analyses (Fig 1) suggest that *C. avellaneus* is closely related to *C. congolensis* and *C. nigrescens*. The latter two species are monophyletic with maximum support (ML-bs = 100%) and sister (ML-bs = 98%) *to C. avellaneus*.The subclade formed by these three species is sister (ML-bs = 79%) to a strongly supported clade (ML-bs = 100%) that is entirely composed of yellow to brown North American and European species. Together with a subclade composed of three specimens of *C. subcyanoxanthus*, all of these species compose subg. *Parvocantharellus* Eyssart. & Buyck. In Buyck et al. (2014), the latter taxon was included in subg. *Parvocantharellus* although without support. In the present analysis, this previously proposed delimitiation of subg. *Parvocantharellus* now receives near significant support (ML-bs = 69%).

Taxonomic results

Cantharellus avellaneus Pat., *Bull. Mus. natl. hist. nat. (Paris)* 30: 412. 1924.
Figs 2-8

Original description (freely translated from French) "Centrally stipitate, fleshy. Pileus convex-plane, with incurved margin, light brownish, irregular, lobed, ca 3 cm diam. Gills decurrent, fold-like, rather spaced, forked, darker brown than the cap, mouse gray upon drying, connected by transversal veins. Stipe central, 2 cm long, curved at the base, massif, dirty whitish, cylindrical, 6 millimeter wide. Spores abundant, not colored, cylindrical, 8-10 × 4 μm."

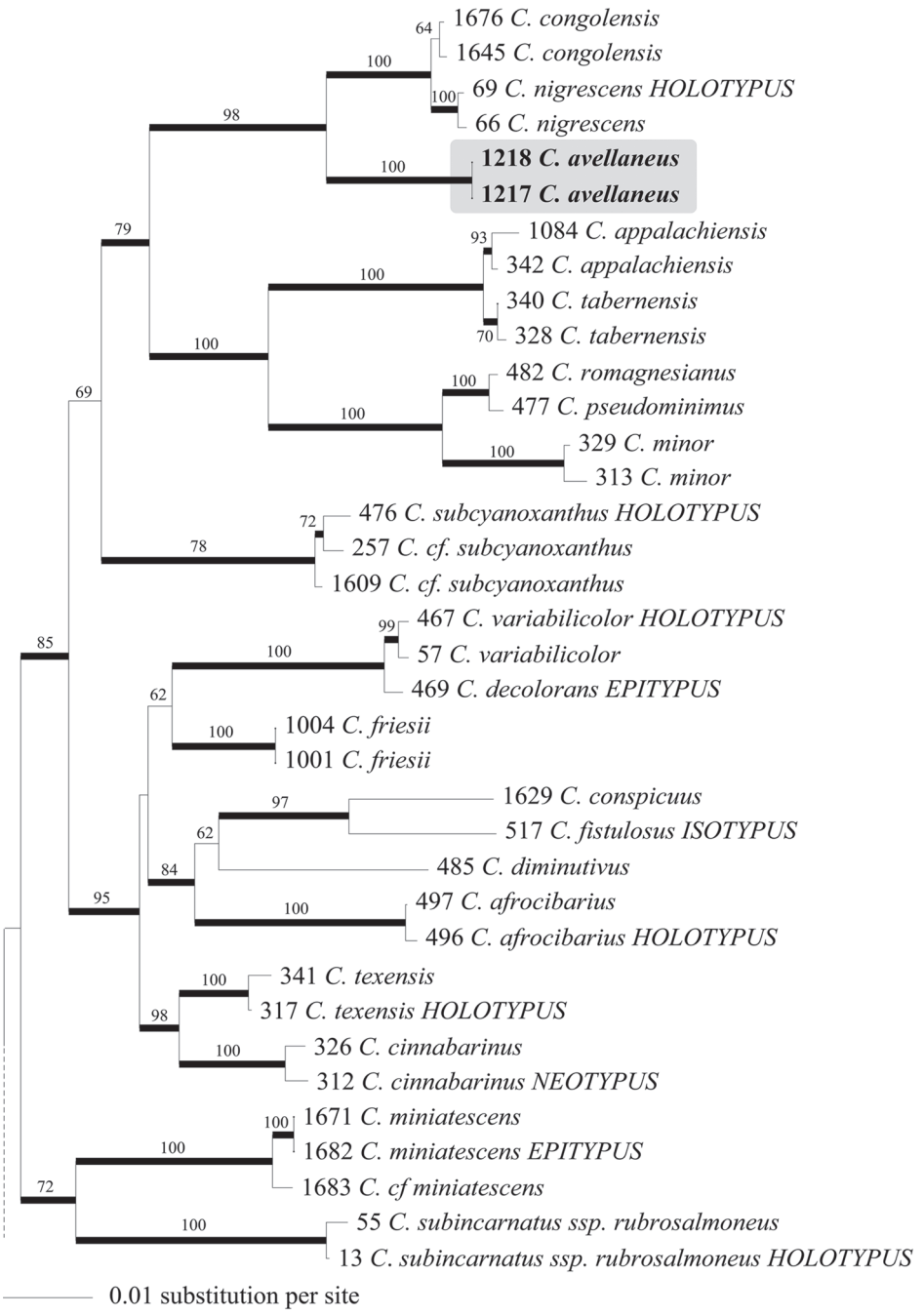
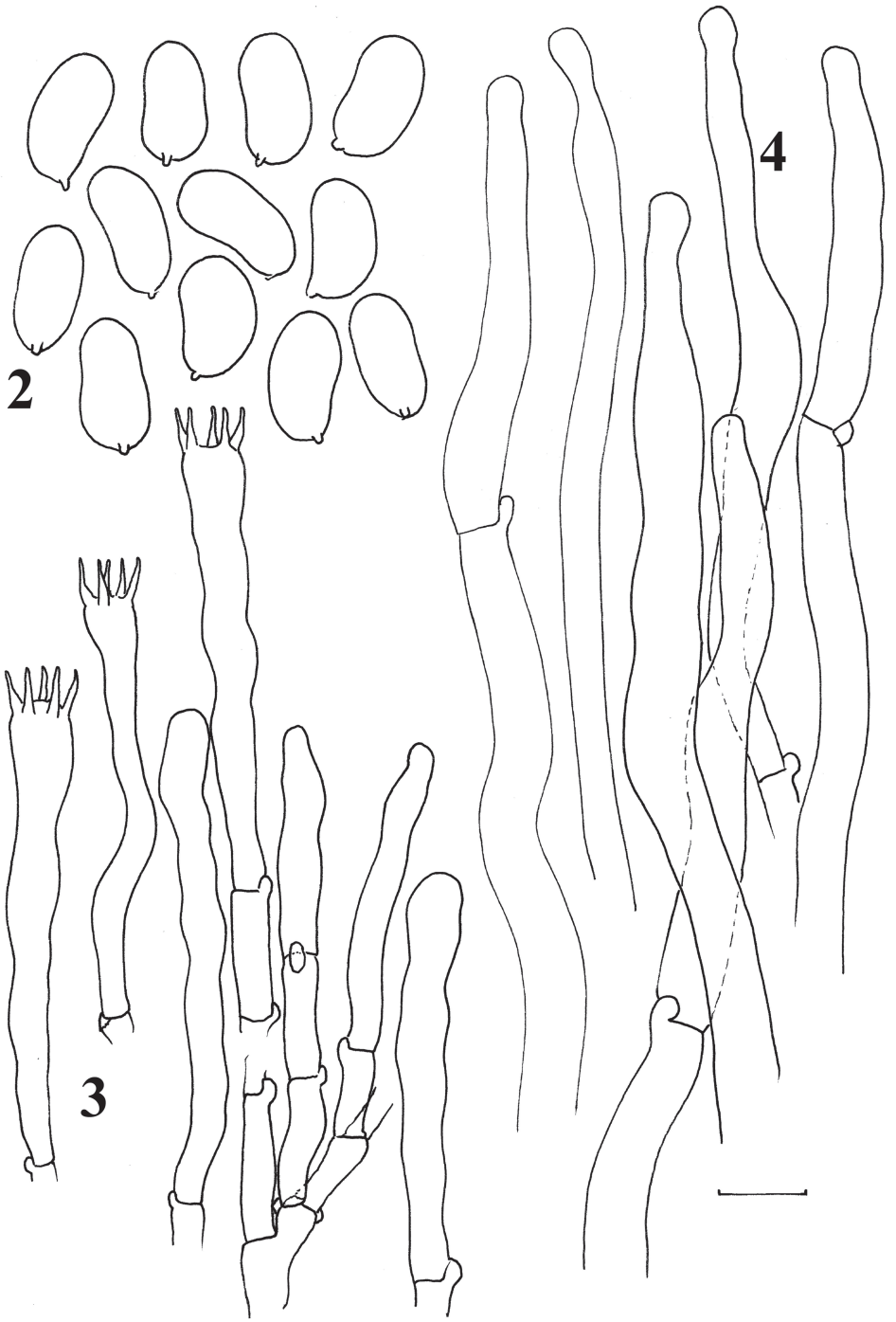


Fig. 1. Part of the most likely tree (-ln = 23582.653) inferred by phylogenetic analyses of the 4 locus-96 taxa alignment. Branches in bold received significant bootstrap values (≥ 70%), which are reported along branches. Extractions 1217 and 1218 were obtained from different fruiting bodies, but generated identical sequences.



Figs 2-4. *Cantharellus avellaneus* (epitypus). **2.** Spores. **3.** Basidia and basidioles. **4.** Hyphal extremities of the pileipellis. Drawings Buyck. Scale bar = 10 µm, but only 5 µm for spores.

Fig. 5. *Cantharellus avellaneus* (epitypus). Detail of fresh fruiting body. Photo E. Randrianjohany.

Fig. 6. *Cantharellus avellaneus* (epitypus). Detail of fresh fruiting body. Photo E. Randrianjohany.

Fig. 7. *Cantharellus avellaneus* (epitypus). Detail of fresh fruiting body. Photo E. Randrianjohany.

Fig. 8. *Cantharellus avellaneus* (epitypus). Exsiccatum showing the typical mouse gray color of the dried hymenophore. Photo B. Buyck.

Epitype description

Pileus 30-70 mm, first plane-convex with strongly inrolled margin, then becoming largely depressed in the center; margin entirely incurved, sometimes fissured in age, irregularly undulating or radially plicate. The surface wet greasy, glabrous, at first dark grayish brown, then becoming tinged with pale yellowish brown or even having greenish hues. **Hymenophore** composed of strongly decurrent, well-differentiated gill-like folds attaining 2-3mm in height, unequal, some sparsely-forked, not interveined, in age with faintly anastomosing transversal veins at their base, cream to pale grayish-pinkish brown, then becoming rapidly and entirely a darker bluish gray. **Stipe** 20-50 × 5-7(10) mm, equal or slightly attenuate at the base, smooth, off-white to pale yellowish, then brown. **Flesh** thick, pale white, when cut turning rapidly pink then slowly dark gray to black. **Spore print** white.

Spores narrowly ellipsoid to almost elongate, (7.7)8.0-*8,39*-8.8(9.0) × (3.7)3.9-*4.19*-4.5(4.6) µm , Q=(1.7)1.8-*2.01*-2.2(2.4), smooth. smooth, filled with one to numerous oily inclusions. **Basidia** mostly 50-65 × 6-8(−9) µm, clavulate, often sinuate in outline, (3-)5(-6)-spored; basidiola slender, subcylindrical, sinuate, becoming tardily clavate. **Subhymenium** filamentous, of slender, strongly septate, hyphal cells, 3-4 µm wide similar to the basidium base. **Cystidia** none. **Pileipellis** a loose cutis of ramifying, thin-walled hyphal extremities, measuring mostly 5-9(12) µm diam., having widely spaced septa; terminal cells subcylindrical and hardly differentiated from the subapical ones, often very long and tapering or slightly constricted subterminally and subcapitate, some containing a brown diffuse pigment; no incrusting pigments observed at the surface but present in lower tissues. **Clamp connections** present everywhere, small.

MADAGASCAR. **Toamasina Prov**., near Antandroroho village, 16 km West of Mahanoro, on unconsolidated sand bordering the Amparafana littoral forest under three co-dominating tree species, viz. *Uapaca littoralis, U. thouarsii* (Phyllantaceae) and *Leptolaena multiflora* (Sarcolaenaceae), E. Randrianjohany 13.144 (PC 0713845, **epitypus hic designatus**).

Additional examined material: MADAGASCAR. **Mahajunga Prov**., Ananalava district, Maromandia, in the sandy soil, January, 14°13′S - 48°5′E, leg. M. Decary (holotype, PC 0084964).

DISCUSSION

There is little doubt that our specimen corresponds to Patouillard's species. Growing in exactly the same habitat (sandy soil) but now on Madagascar's east coast, it shares with the holotype the brown pileus and the distinct gill folds that, although quite pale at first, become rapidly comparatively dark in color and, upon drying, turn to mouse gray (Fig. 8). Eyssartier (2001) revised the holotype and measured spores as 7.5-*8.63*-10 × 3.5-*3.95*-4.5 µm and Q = 1.78-*2.19*-2.43, which is nearly exactly the measurements we obtained for our specimen.

As there exist two places called Maromandia in Madagascar, we hesitated for some time which of these two corresponded to the holotype locality: either the one in Mahajunga Province in Northeastern Madagascar, or the one on the Masoala peninsula in Antsiranana Province on the east coast, a bit more to the north from where the here presented specimen was collected. Of course, it was tempting to

admit that the closest locality was the most probable location, but a search among Decary's publications accessible via the French National Library (at http://Gallica.bnf.fr) showed that Decary had repeatedly been visiting the region of Maromandia in the northeastern part of Madagascar, including the period that he collected the *C. avellaneus* holotype (Decary, 1923), while botanic inventories of the ectomycorrhizal Sarcolaenaceae in Madagascar demonstrated that potential hosts for these chanterelles were abundantly present in both localities (Lowry II *et al.*, 2002; Soulebeau *et al.*, 2016).

C. avellaneus differs from the African *C. congolensis* (a name originally designating a rain forest species, but still widely applied to woodland collections for the moment) and the Malagasy, eucalypt-associated *C. nigrescens* by the absence of a strong blackening reaction upon handling or with age and the distinct gill-like folds having only a faint interstitial venation. Patouillard's species also presented a more greasy-humid cap surface, but it is possible that this relates to the quite advanced state of some fruiting bodies. *C. avellaneus* does present a color change of its context that is reminiscent of *C. congolensis* but the reaction is much less energetic and Patouillard's species cannot be considered to be a "blackening" chanterelle as its fruiting bodies do not become completely black with age. Under the microscope, however, Patouillard's species strongly resembles both other chanterelles and shares with them near identical features of basidia and pileipellis [compare with fig. 137 for *C. nigrescens* in Ariyawansa *et al.* (2015)].

Our phylogeny (Fig. 1) places *C. avellaneus* with very high support in *Cantharellus* subg. *Parvocantharellus* sect. *Congolenses* Heinem. where it fits perfectly given the above-mentioned morphological resemblances and the clamped, thin-walled hyphae of the pileipellis (Buyck *et al.* 2014).

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