Cantharellus coccolobae sp.nov.and Cantharellus garnieri, two tropical members of Cantharellus subg.Cinnabarinus.

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6 authors, including:

Pierre-Arthur Moreau
Université du Droit et de la Sa…

Hofstetter Valerie
Agroscope

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Cantharellus coccolobae sp. nov. and *Cantharellus garnieri*, two tropical members of *Cantharellus* subg. *Cinnabarinus*

Bart BUYCK[a], Pierre-Arthur MOREAU[b], Régis COURTECUISSE[b],*
Alejandro KONG[c], Mélanie ROY[d] & Valérie HOFSTETTER[e]

[a]*Muséum National d'Histoire Naturelle, Dépt. Systématique et évolution,*
CP 39, ISYEB, UMR 7205 CNRS MNHN UPMC EPHE, 12 Rue Buffon,
F-75005 Paris, France ; email: buyck@mnhn.fr

[b]*Université de Lille, LSVF, EA 4483. BP 83, F-59006 Lille cedex, France*

[c]*Laboratorio de Biodiversidad, Centro de investigación en Ciencias Biológicas,*
Universidad Autónoma de tlaxcala, km 10.5 carretera San Martín
Texmelucan-Tlaxcala, San Felipe Ixtacuixtla, Tlaxcala, 90120 México

[d]*Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, CNRS,*
UMR5174, 118 route de Narbonne, 31062 Toulouse Cedex,France

[e]*Department of plant protection, Agroscope Changins-Wädenswil Research Station*
ACW, Rte De Duiller, CH-1260 Nyon, Switzerland

Abstract – This paper attributes two more species to *Cantharellus* subg. *Cinnabarinus*: the here newly described *C. coccolobae*, a strict associate of *Coccoloba* species in subtropical and tropical America, and the New Caledonian *C. garnieri*. A multigene analysis places both species in a genus phylogeny and their macro- and microscopic features are illustrated and discussed.

***Coccoloba* / Caribbean / Florida / Mexico / multigene phylogeny / taxonomy**

INTRODUCTION

Cantharellus subg. *Cinnabarinus* Buyck & V. Hofstetter was introduced two years ago (Buyck *et al*. 2014) for a monophyletic assemblage of mostly quite small, yellow, orange, pink or red species, sometimes mixed with lilac-purple or brownish tones, particularly in the cap center, and all having principally thin-walled hyphal endings and abundant clamp connections. At that time, this subgenus was composed of only eight species and it was the only subgenus uniting species from as many as four continents (Europe, North America, Australasia and Africa including Madagascar). Since then, four new taxa have joined the subgenus: the Malagasy *C. variabilicolor* Buyck & V. Hofstetter (in Ariyawansa *et al*. 2015), the Chinese

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C. phloginus S.C. Shao & P.G. Liu (in Shao *et al*. 2015), the American *C. corallinus* Buyck, Justice & V. Hofstetter (in Buyck *et al*. 2016c) and even a cyphelloid species from Japan, *C. cyphelloides* Suhara & S. Kurogi (2015).

In this paper, the authors add two more species to the subgenus using a multigene sequence dataset. The first species is the New Caledonian *C. garnieri* Ducousso & Eyssart. (*ut C. garnierii*) originally placed in *Cantharellus* subg. *Cantharellus* (Ducousso *et al*. 2004). The second is here newly described from the Caribbean, but with additional collections reported from Mexico and Florida based on the similarity of their morphological features.

MATERIAL AND METHODS

Morphological data. — All sequenced collections were gathered by the first author and collaborators in the past few years. All cited specimens are deposited at the mycological herbarium of the Paris' Natural History Museum (PC) unless indicated otherwise. Microscopic features were examined and sketched by B. Buyck using a camera lucida setup. Original drawings for all elements of the hymenium or pellis were made at × 2400. All microscopic observations and measurements were made in ammoniacal Congo red, after a short aqueous KOH pretreatment to improve tissue dissociation and matrix dissolution. Measurements of basidiospores cite length, width and length/width ratio (Q) in the following format : (minimum measured-) mean minus stand.dev. – *mean value* – mean plus stand.dev. (-maximum measured); the spore measurements are based on 20 spores/collection.

Phylogenetic data. — For phylogenetic purposes we produced sequence data for the four genes (mitSSU, nucLSU, RPB2 and *TEF*-1) used in the *Cantharellus* phylogeny by Buyck *et al*. (2014). Newly produced sequences are shown in Table 1. Fungal genomic DNA isolation, amplification, sequencing and subsequent phylogenetic analyses were performed as in Buyck *et al*. (2016a).

Table 1. Voucher table showing additional or replaced *Cantharellus* sequence data compared to the voucher table in Buyck *et al.* 2016a

Taxon	*Voucher*	*Provenance*	*Herbarium*	*Genbank accession numbers*			
				mitSSU	*nucLSU*	*RPB2*	*TEF1*
C. coccolobae	1065/RC 11.25	Guadeloupe	PC 0142434	KX857114	KX857088	KX856992	KX857020
C. coccolobae	1064/RC 14.24	Guadeloupe	PC 0713853	KX857115	KX857089	KX856993	KX857021
C. garnieri	1020/BB 09.024	New Caledonia	PC 0084860	KX857111	KX857085	KX856989	KX857017
C. garnieri	1021/BB 09.033	New Caledonia	PC 0084861	KX857112	KX857086	KX856990	KX857018
C. garnieri	1030/BB 09.283	New Caledonia	PC 0084867	KX857113	KX857087	KX856991	KX857019
C. guyanensis	1501/MR G3026	Guyane	PC 0713383	KX857120	KX857094	KX856998	KX857060
C. guyanensis	1517/MR G3183	Guyane	PC 0713513	KX857121	KX857095	KX856999	KX857061

RESULTS

Phylogenetic results

Phylogenetic analyses (Fig 1) suggest that *C. coccolobae* is closely related to *C. texensis* and *C. cinnabarinus.* These three species are grouped in a monophyletic clade with maximum support (ML-bs = 100%) and *C. coccolobae* is placed as sister clade (ML-bs = 72%) of *C. texensis.* The position of the subclade formed by these three species with respect to other reddish species of subg. *Cinnabarinus* (the European *C. friesii*, the American *C. corallinus* and the New Caledonian *C. garnieri*) is not resolved. In the present analysis, the delimitiation of subg. *Cinnabarinus* as proposed by Buyck *et al*. (2014) is highly supported (ML-bs = 95%).

Taxonomy

Cantharellus coccolobae Buyck, Moreau & Courtecuisse ***sp. nov.*** **Figs 2-4, 8-14**

Mycobank: MB818375

Diagnosis: Differs from the other known species in subg. *Cinnabarinus* by the presence of irregularly undulating, partly or predominantly thick-walled hyphal extremities in the pileipellis, often long basidia and the apparently specific association with *Coccoloba* species. Spores ellipsoid, (7.9)8.3-*8.78*-9.3(9.8) × (4.8)5.3-*5.59*-5.9(6.0) µm, Q = (1.3)1.4-*1.58*-1.7(1.9).

Etymology: refers to the host association of this chanterelle

Holotype: GUADELOUPE. Saint Francois, La Baie Olive, under *Coccoloba swartzii* in rocky slope bordering floodplain, 06 Aug. 2011, R. Courtecuisse RC/Guad 11.025 (holotypus LIP, isotypus PC0142434)

Basidiomata gregarious, sometimes with many individuals, small but rather robust and fleshy. **Pileus** 20-35 mm diam., convex but then flattened to slightly depressed in the center; surface smooth to finely tomentose-fibrillose toward the margin, dull, almost with greasy aspect when wet, salmon to red or reddish pink, slightly hygrophanous and discoloring rapidly to pale pink or pinkish orange when drying out; margin strongly incurved when young, remaining often oriented downward in age, sometimes more or less concentrically zoned, yellowing upon manipulation. **Hymenophore** decurrent, composed of thick veins, without well-developed gill folds, forked and not transversally interveined in between, sometimes locally with hardly visible veins or nearly smooth, particularly closer to or on the stipe apex and near the cap margin, young sometimes a very pale pink, at maturity a beautiful salmon orange. **Stipe** 20-35 × 5-8 mm, subcylindrical to more inflated toward the base, smooth, pale pink close to the hymenophore, more orange to yellowish toward the base, distinctly yellowing upon handling or when cut, particularly in the lower half, compact. **Flesh** firm, white, reddish pink close to the cap surface, yellowing in the lower stipe half. **Odor** typical, fruity. **Taste** first mild, then slowly acquiring a distinct acridity on the tip of the tongue. **Spore print** not obtained for the holotype, but off-white in dried faint spore deposit for RC/Mart12.006.

Spores ellipsoid, (7.9)8.3-*8.78*-9.3(9.8) × (4.8)5.3-*5.59*-5.9(6.0) µm, Q = (1.3)1.4-*1.58*-1.7(1.9), smooth. **Basidia** very long and slender, 80-120 × 9-11 µm, predominantly 5(-6) spored, with a short apiculus. **Subhymenium** distinctly

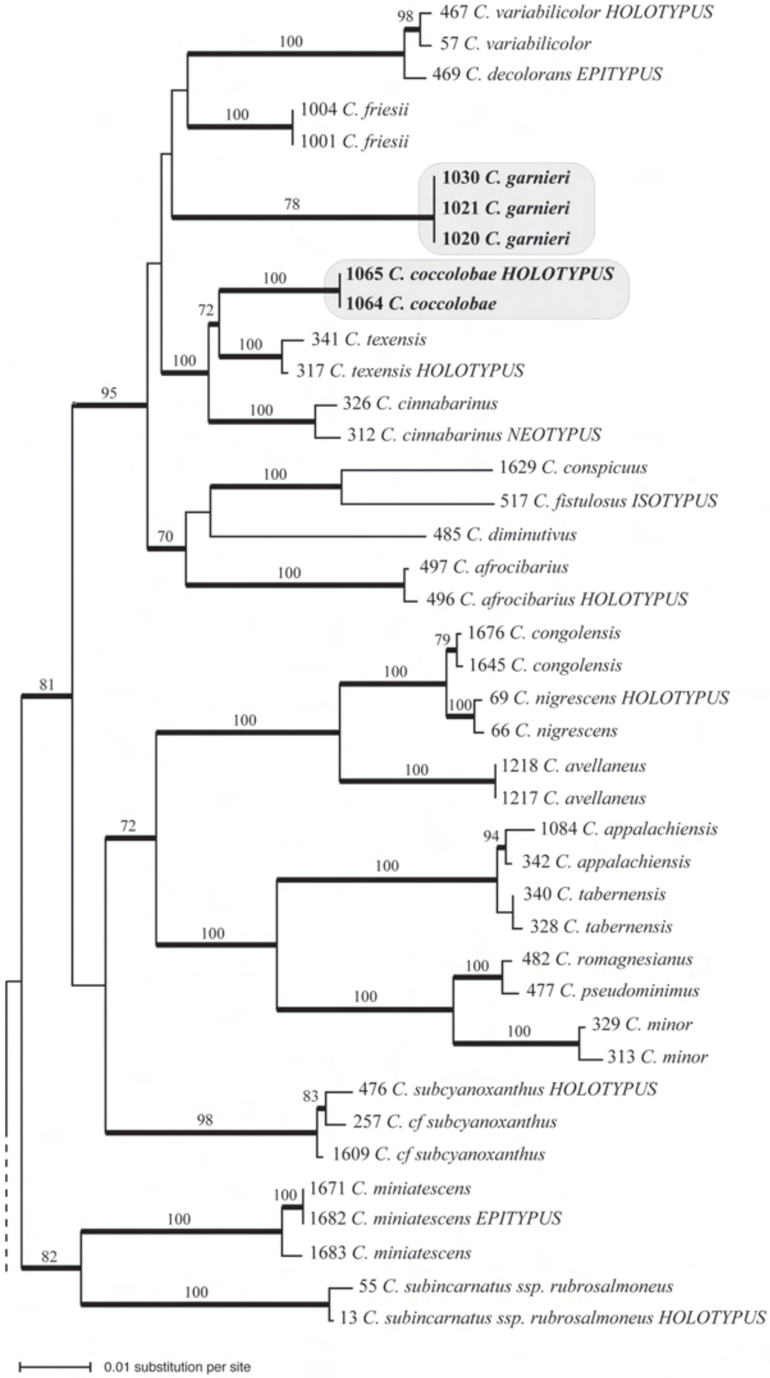
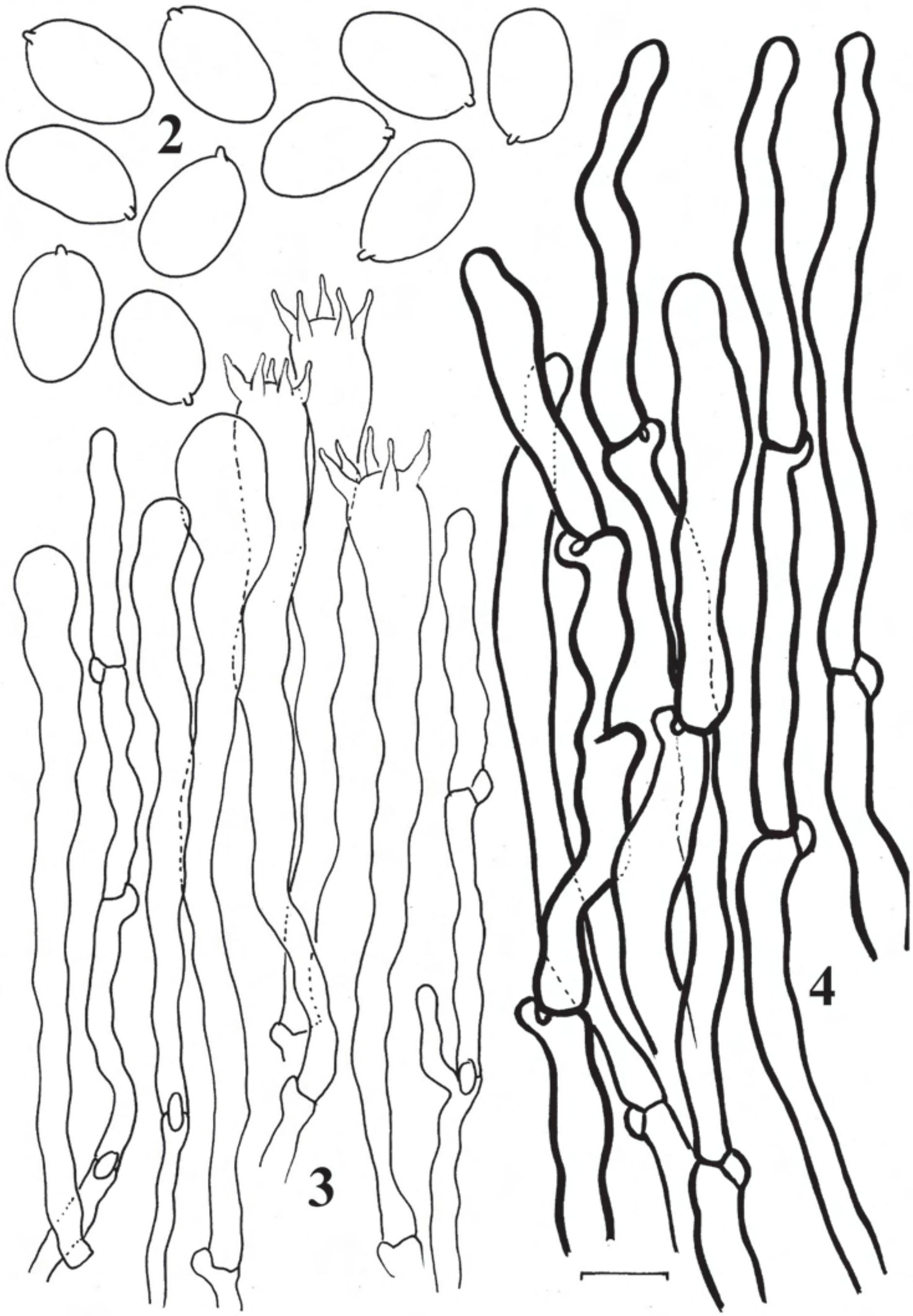


Fig. 1. Part of the most likely tree (-ln = 23483.176) inferred by phylogenetic analyses of the 4 locus-101 taxa alignment. Branches in bold received significant maximum likelihood (ML) bootstrap (bs) values (ML-bs ≥ 70%), which are reported along branches.



Figs 2-4. *Cantharellus coccolobae* (holotypus). **2.** Spores. **3.** Basidia and basidiola. **4.** Hyphal extremities of the pileipellis. Scale = 10 µm, but only 5 µm for spores. Drawings B. Buyck.

filamentous, composed of very slender and long, cylindrical cells similar or narrower in diam. compared to the base of the basidia. **Cystidia** none. **Pileipellis** composed of rather short-celled, thin- to thick-walled hyphal extremities, 4-10 (-20) µm diam., here and there subapically ramifying; terminal cells variously shaped, more or less subcylindrical to clavate, but mostly irregularly undulating or sinuous, 30-60(90) µm long. **Clamp connections** abundant in all tissues and very obvious.

Other examined material:

Guadeloupe. Marie-Galante, in sandy soil on rocky roadside in coastal forest with *Coccoloba uvifera*, 11 Aug. 2011, P.-A. Moreau Guad11-14 (LIP); Saint Francois, La Baie Olive, in rocky slope bordering floodplain under *Coccoloba swartzii*, 06 Aug. 2011, R. Courtecuisse RC/Guad 11.024 (LIP)

Martinique. Le Vauclin. Near beach Anse Grand Macabou, in sandy soil under *C. uvifera*, 8 Aug. 2012,R. Courtecuisse RC/ Mart 12 (LIP); near beach Le Marin, Anse Four à Chaux (Cap Macré), in sandy soil under *Coccoloba uvifera*, 15 Aug. 2012, A. Favel in P.-A. Moreau Mart12-74 (LIP).

Not yet sequenced, but putative representative material:

Mexico. Yucatan. Holchit, Ría Lagartos Biosphere Reserve, 21°37´11.3”N - 88°06´16.2”O W. 3-5 m alt., in Sand Dune Shrubland, “Matorral de dunas costeras”, sandy soil under *Coccoloba uvifera*, 5 Sept. 2012, A. Kong 12-754 (TLXM); El Cuyo, Tizimin, Ria Lagartos Biosphere Reserve, 21°31´16.6”N/-87°42´19.3” W, 3-5 m alt., in Sand Dune Shrubland, “Matorral de dunas costeras”, sandy soil under *Coccoloba uvifera*, G. Galindo 14-05-14 (TLXM)

United States of America. Florida. North Miami, in a park just off the main walking path, in thick clusters under *Coccoloba diversifolia* (Pigeon plum tree), 2014, M. Smiley leg. (PC 0142435); Bonita Beach, north of Naples, forming thick clusters in the sandy soil near the beach, in association with *Coccoloba uvifera*, 16 Aug. 2016, M. Smiley leg. (PC 0142436)

Commentary: This species has typically been identified in the past as “*C. cinnabarinus*” but appears to be a strict associate of *Coccoloba* species. It was first mentioned from the Lesser Antilles by Pegler (1983, as “*C. cinnabarinus*”). The description by Pegler confirms the slight acridity of the flesh and the larger size of the spores when compared to *C. cinnabarinus* [spores of the neotype for the latter were given in Buyck *et al*. (2011) as (6.5) 6.7-*7.14*-7.6(8.1) × (3.7)3.8-*4.25*-4.7(5.2) mm, Q 5 (1.5)1.57-*1.69*-1.80(1.9)]. Other features in the description by Pegler are deviating from our analysis and should be verified: e.g. short basidia and thin-walled hyphal extremities in pileipellis. Obviously, in the context of a book on all gilled fungi, a 1 µm thick cell wall can be considered ‘thin’, but in the strict context of *Cantharellus*, this corresponds to what is commonly designated as being thick-walled. Nevertheless, the Mexican collections also have predominantly thin-walled (<0.5 µm) terminations in the pileipellis with only few cells being distinctly thick-walled [cell walls 0.8-1.2(-1.6) µm thick].

As for the length of the basidia, we have also observed noticeably shorter basidia in the specimens from Martinique.

Cantharellus cinnabarinus has a very loose pileipellis composed of thin-walled elements with many free clavate terminal cells some of which are more or less thick-walled, while the recently described *C. texensis* and *C. corallinus* (Buyck *et al*. 2016c) are easily distinguished because of their dense cutis composed of thin-walled, regularly cylindrical hyphal extremities with more distant septa and few free endings. Both latter species always possess well-developed gill-folds and are typically less robust and have a longer, more slender stipe compared to *C. coccolobae*. Yet, specimens in RC/Guad 11.024 (from Guadeloupe) differ from the other collections in an equally less robust appearance and they also lack the distinctly yellowing context, but spore measurements gave very similar results: (6.7)7.9-*8.51*-9.1(9.8) × (4.8)5.1-*5.48*-5.9(6.2) µm, Q = (1.3)1.4-*1.56*-1.7(1.9). Fruiting bodies of this collection also exhibit a zonate structure of the context very comparable to the context structure of *Multifurca* (Russulaceae) - see Buyck *et al.* 2008). A similar zonation can sporadically also be observed in some tropical African *Cantharellus* (Buyck, pers. obs.) but appears to have no particular taxonomic or diagnostic importance for species recognition in this genus.

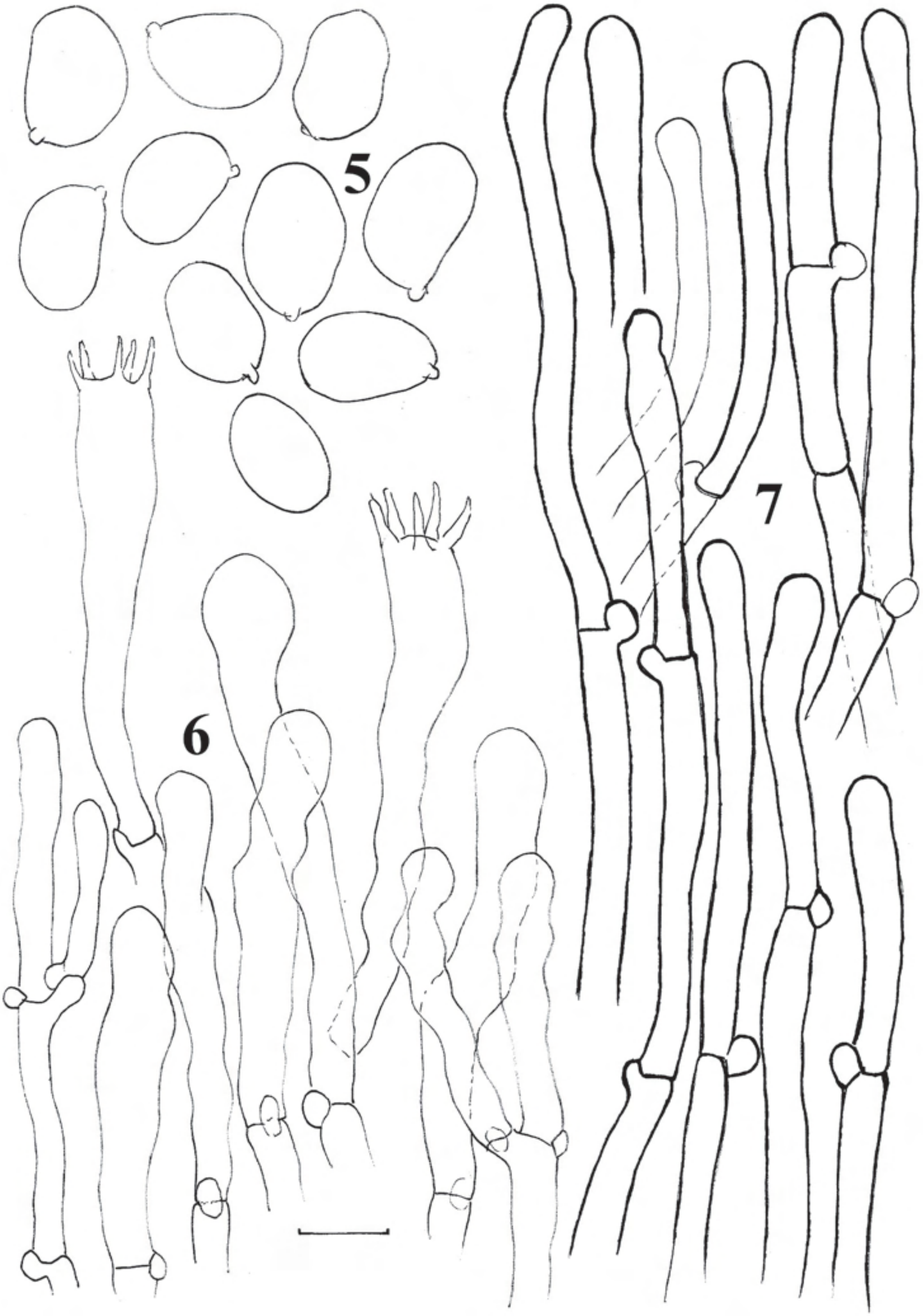
Compared to the other small, reddish species of subg. *Cinnabarinus*, *C. coccolobae* stands out because of the frequently long basidia, the filamentous subhymenium and it may produce sometimes specimens with a much smoother hymenophore (Fig. 9), even to an extent that some can almost be considered as 'smooth chanterelles' (Buyck 2014). These characters are often observed in chanterelles that occupy more basal positions in the main infrageneric monophyletic clades (Buyck *et al.* 2014) as equally suggested by a partial ITS-LSU analysis (Buyck *et al.* 2016b).

Cantharellus garnieri Ducousso & Eyssart., *Cryptogamie, mycol.*25(2): 140. 2004.
Figs 5-7, 15

A detailed description was provided in the original publication. Our specimens agree in every respect with the original description, except for the strongly veined-anastomosed hymenophore (Fig. 11). Spores measurements for such specimens are identical to those in the original description: (7.3)7.6-*8.28*-8.9(9.8) × (5.2)5.2-*5.63*-6.0(6.5) µm, Q = (1.3)1.4-*1.47*-1.5(1.6) for BB09.283 versus (7.5) 8-*8.34*-9.25 (10) × (5) 5.25-*5.68*-6 µm. Q = 1.33-*1.52*-1.83 for the holotype collection (Ducousso *et al.* 2004)

Examined material: NEW CALEDONIA. Northern Prov., Koniambo, ca 720 m alt., on ultramafic soil under *Nothofagus balansae*, 17 March 2009, leg. Buyck, B., Eyssartier G., Hofstetter, V. & M. Ducousso, in Buyck 1020/09.024 (PC 0084860), 1021/09.033 (PC 0084861), 1022/09.053 (PC 0084862); Ile des Pins, Le Bagne, under *Acacia*, 3 April 2009, Buyck, B., Eyssartier G., Hofstetter, V. & M. Ducousso legunt, in Buyck 1030/09.283 (PC 0084867); Koniambo, forest near Trazy entry with *Nothofagus codonandra*, 9 April 2009, legunt Buyck, B., Eyssartier G., Hofstetter, V. & M. Ducousso, in Buyck 1032/09.355 (PC 0084868).

Commentary: This species was described as having 'distinctly thickened cell-walls' in the pileipellis, but the description specified nevertheless a thickness of merely 0.5 µm, which will surely be perceived as absolutely thin-walled by most people. Also the first author tends to consider such cells still as 'thin- to slightly thick-walled' when compared to other chanterelles, but hyphal terminations are nevertheless distinctly refringent, more so than in typically thin-walled species, such as *C. texensis* for ex. Because of the mention of such "thick-walled" hyphal extremities, this species was originally placed in subg. *Cantharellus* (Ducousso *et al.* 2004). The hymenophore of this species was originally described as not or only weakly interveined. Our collection 09.283 (Fig. 14) clearly shows that hymenophore development is quite variable (as in *C. coccolobae*) and may produce gill folds which are occasionally strongly interveined.



Figs 5-7. *Cantharellus garnieri* (Buyck 09.283) **5.** Spores. **6.** Basidia and basidiola. **7.** Hyphal extremities of the pileipellis. Scale = 10 µm, but only 5 µm for spores. Drawings B. Buyck.

Fig. 8. *Cantharellus coccolobae* (holotype). Note the yellowing of the lower stipe. (Photo R. Courtecuisse).

Fig. 9. *Cantharellus coccolobae* (P.-A. Moreau Guad11-14). Note the poor hymenium development and absence of distinctly yellowing tissues. (Photo P.-A. Moreau).

Figs 10-13. *Cantharellus coccolobae*. Field habitat and specimens from Florida (on the left, associated with *C. diversifolia,* photos M. Smiley) and Mexico (on the right, associated with *C. uviferae*, photos A. Kong). Note the yellowing lower stipe and strongly decurrent, well-developed hymenophore with abundant interstitial anastomosing veins.

Fig. 14. *Cantharellus coccolobae*. Fied aspect of clustered fruiting bodies. Not the greasy, smooth cap surface in the fresh, well-hydrated specimens (Photo M. Smiley).

Fig. 15. *Cantharellus garnieri*. (Buyck & Eyssartier 09.283, photo G. Eyssartier).

Cantharellus garnieri (originally published as '*garnierii'*) lacks reddish, pink or bright orange tinges and is a more yellowish to yellowish orange species in a group of predominantly red-pink-orange species (Fig. 1). It can easily be confused with the equally yellow and closely related *C. wellingtonensis* McNabb, originally described from New Zealand (McNabb 1971), but also present in New Caledonia (Buyck, unpubl.).

The original publication did not mention host trees for *C. garnieri*. Our data suggest that is not host specific as it appears to associate at least with both *Acacia* (Fabales) and *Nothofagus* (Fagales) in New Caledonia.

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