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Article

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The type species of *Tetrapyrgos* and *Campanella* (Basidiomycota, Agaricales) are redescribed and epitypified

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Abstract

Tetrapyrgos atrocyanea, the type species of *Tetrapyrgos*, and *Campanella buettneri*, the type species of *Campanella*, are redescribed and epitypified based on recently collected material from Madagascar and Príncipe, respectively, supported with morphological and LSU, ITS sequences data. Line drawings, colour photographs, and comparisons with similar taxa are provided.

Key Words – agarics – *Campanella buettneri* – fungal diversity – taxonomy – *Tetrapyrgos atrocyanea*

Introduction

There has been renewed interest in clarifying the generic boundaries of *Tetrapyrgos* E. Horak (1987) and *Campanella* Hennings (1895). The morphological distinctions between these two genera and the ways they have been treated taxonomically by various workers were succinctly documented by Honan et al. (2015). However, they noted that distinguishing *Tetrapyrgos* from *Campanella* was problematical because of the lack of recently collected specimens of their type species upon which comprehensive morphological and molecular redescriptions can be based. The holotype specimen of the type species of *Tetrapyrgos* (*Pterospora atrocyanea* Métrod) was collected in 1935 in Madagascar, and is stored in alcohol (PC), rendering it of limited molecular phylogenetic value. The holotype specimen

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of the type species of *Campanella* (*C. buettneri* Henn.) was collected in 1892 in Togo, is also stored in liquid (B), and of limited value for molecular analyses. Our recent fieldwork in Madagascar and the West African islands of São Tomé and Príncipe (approx. 700 miles from Togo) have yielded specimens that we recognize as *Tetrapyrgos atrocyanea* and *Campanella buettneri*, respectively. The purpose of this paper is to redescribe these two important type species, based on morphological characteristics of these recent specimens which we designate as epitype material, and to provide ITS and nLSU sequences to aid in distinguishing the species from others, and for future research. Determining whether *Tetrapyrgos* and *Campanella* represent a single, morphologically variable genus is beyond the scope of this contribution.

Materials and Methods

Morphological observations

Fresh material of *Tetrapyrgos atrocyanea* was collected in Madagascar in Febuary 2014, and fresh material of *Campanella buettneri* was collected in São Tomé and Príncipe April 2006 and 2008. Macromorphological notes and photographs were documented soon after collection, before drying the basidiomes for transport and herbarium storage. Colour terms and notations in parentheses are those of Kornerup & Wanscher (1978). Micromorphological analyses were performed using dried material rehydrated in 100% ethanol followed by Melzer's reagent, or Congo Red Solution and 3% KOH, and documented using a Nikon Optiphot-2 compound microscope fitted with a drawing tube. Because of the unusual tetrahedral shape of basidiospores of *Tetrapyrgos*, their length and width were determined by measuring the sides of a quadrilateral into which each spore would fit. Spore statistics were calculated as: x_m, the arithmetic mean of the spore length by the spore width (± standard deviation) from n spores measured in a single specimen; Q, the quotient of spore length and spore width in any one basidiospore indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, the number of spores measured per specimen; s, the number of specimens studied. Specimens are deposited in the H.D. Thiers Herbarium at San Francisco State University (SFSU).

Molecular methods

Total genomic DNA was extracted from dried material using the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, MO) following the manufacturer instructions. PCR protocols followed those outlined in Perry et al. (2007). The nuclear ribosomal internal transcribed spacer region (ITS) and ribosomal large subunit (nLSU) were amplified using primer pairs ITS1-F/ITS4 (Gardes and Bruns 1993; White et al. 1990) and LROR/LR7 (Moncalvo et. al. 2000), respectively. Amplification products were cleaned using the Exo-SAPit kit (Affymetrix, Santa Clara, CA), and sent to ELIM Biopharmaceuticals (Hayward, CA) for sequencing. Resulting sequencing products were edited, assembled, aligned and compared to top BLAST hits in Geneious 9.0 (Biomatters Ltd., Auckland, New Zealand). All sequences generated as part of this study have been deposited in GenBank (accessions MF075136 – MF075139).

Taxonomy

Tetrapyrgos atrocyanea (Métrod) E. Horak, Sydowia 39: 102. 1987 (1986). Figs. 1, 2a–e

Basionym: *Pterospora atrocyanea* Métrod, Prod. Fl. Mycol. Madag. 3: 140. 1949.

Holotype – MADAGASCAR. Antananarivo (as Tananarive), in a pasture in a government park, 16 Dec. 1934, coll. by R Decary, No. 1642 (PC).

Epitype *hic designari* – MADAGASCAR. Region Analamanga, Antananarivo, Parc Botanique de Zoologique, near the garden of Crops Wild and Relatives, S18˚55.530', E47˚31.350', elevation 1270 m, 8 Feb. 2014, coll. by DS Newman and JE Shay, *JES 216* (MF075139–LSU, MF075137–ITS, SFSU).

MycoBank: MB 131349; Facesoffungi number: 03427

Description of epitype material:

Pileus 5–25 mm diam, convex to plano-convex, centrally slightly depressed in age; margin striate; surface dull, dry, felted; disc initially brownish grey (5C1–3), developing dark bluish grey tones in age, margin initially white, becoming pinkish buff to orangish white (5A3) in age, staining dark bluish grey (20E3–4) in splotches. Context up to 1 mm thick, pliant, tenacious, concolorous with pileus surface, staining dark bluish grey. Lamellae adnate with a decurrent tooth to arcuate, close (19–25) with 2–3 series of lamellulae, moderately broad (1–2 mm), white, often becoming pale orange white (5A2) in age, sometimes staining bluish grey; edges pruinose. Stipe 10–36 × 1–2 mm, central, cylindrical or narrowed downward, tough, pliant, hollow, non-insititious, arising from a flattened pad of bluish black mycelium; surface dull, dry, felted-pruinose; apex white when young, becoming orangish white (5A2) to brownish grey (5E–F3) in age; base brownish grey (5F3) to dark bluish grey (20D–E3–4) to bluish black (20F4–5).

Fig. 1 – Basidiomes of *Tetrapyrgos atrocyanea* (Epitype, JES 216). Scale bars = 10 mm

Photographs by DS Newman

Basidiospores (8–) 10–11.8 × (6.5–) 7–9 µm [x_m = 11.0 ± 0.63 × 7.9 ± 0.75 µm, Q = 1.13–1.77, Q_m = 1.41 ± 0.27, n = 20, s = 1], tetrahedral, hyaline, inamyloid, thin-walled. Basidia 30–40 × 7–8.5 µm, clavate, 4-spored, hyaline. Basidioles subclavate to subfusoid. Pleurocystidia absent. Lamellar edge sterile. Cheilocystidia abundant, 30–50 × 4–5 µm, irregularly cylindrical, densely diverticulate, usually with a knob-like apex; apical knob 5–14 × 3.8–7 µm, broadly obtuse, smooth; diverticula 1.5–5 × 1–3.5 µm, cylindrical to knob-like, obtuse; hyaline, inamyloid, non-gelatinous, thin-walled. Subhymenium non-gelatinous. Pileipellis a well-developed *Rameales*-structure of hyphae 3.5–7 µm diam, irregularly cylindrical, sometimes forked, densely diverticulate; terminal cells repent to erect, similar to the cheilocystidia, typically with a knob-like apex; apical knob 5–16 × 4.5–9 µm, broadly obtuse, smooth; diverticula 1.5–5 × 1.5–3.5 µm, cylindrical to knob-like, obtuse; hyaline, inamyloid, non-gelatinous. Pileus trama of loosely interwoven hyphae 2–5 µm diam, cylindrical to irregular in outline, hyaline, inamyloid, strongly gelatinous. Lamellar trama regular, hyphae 2–5 µm diam, cylindrical to irregular in outline, hyaline, inamyloid, strongly gelatinous. Stipitipellis similar to the pileipellis, a well-developed *Rameales*-structure of densely diverticulate hyphae with apical knobs; stipe cortical hyphae 2.5–5 µm diam, parallel, cylindrical, hyaline, inamyloid, non-gelatinous, thin- to thick-walled; stipe medullary hyphae 3–10 (–12) µm diam, cylindrical, hyaline, inamyloid, non-gelatinous. Clamp connections common in all tissues.

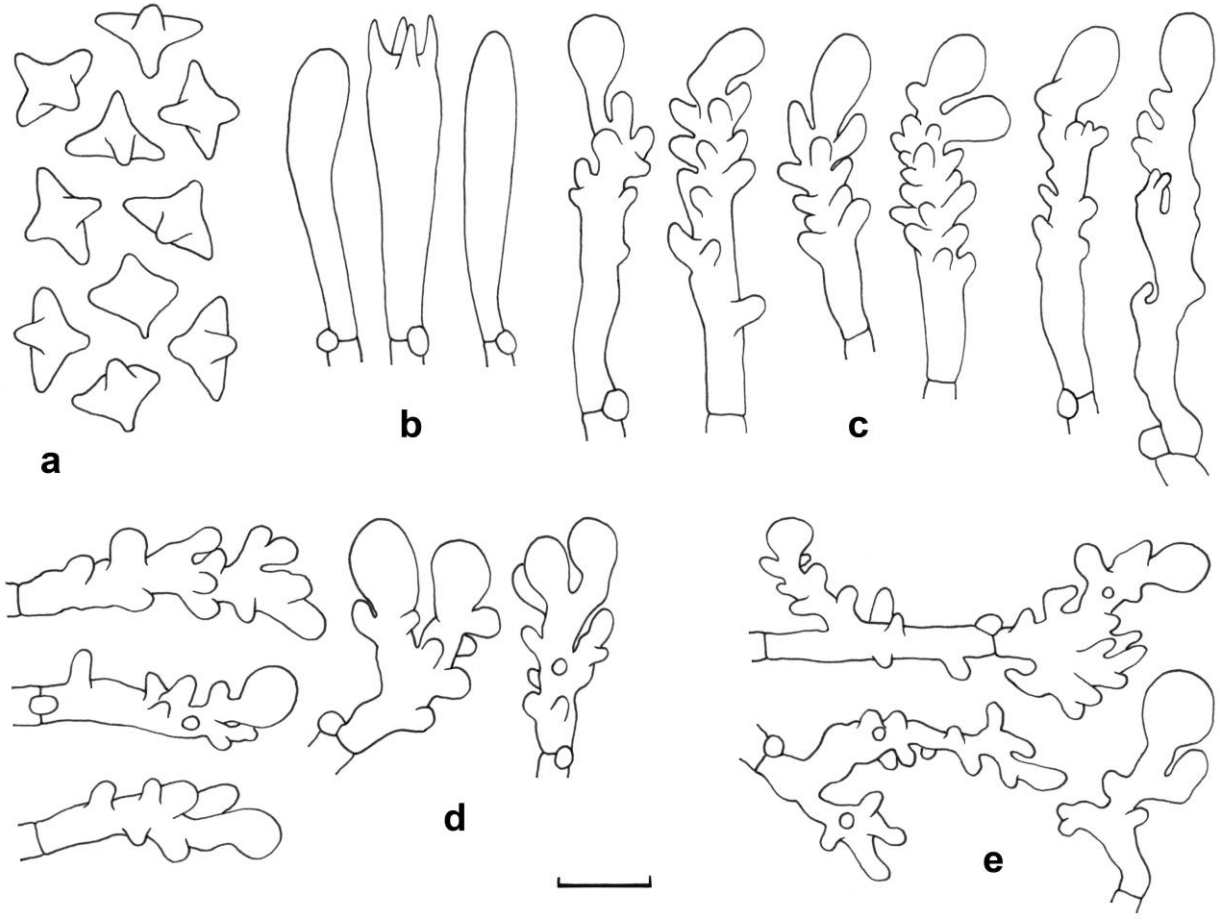

Fig. 2 – Micromorphological features of *Tetrapyrgos atrocyanea* (Epitype, JES 216). a. Basidiospores. b. Basidium and basidioles. c. Cheilocystidia. d. Pileipellis terminal cells. e. Stipitipellis cells. Scale bar = 10 µm. Illustration by DE Desjardin.

Habit, habitat and known distribution – Gregarious, sometimes subcespitose, on woody debris amongst *Casuarina*, *Panicum*, *Galinsoga*, *Commelina* and bamboo, in a botanical garden and zoological park. Madagascar.

Notes – *Tetrapyrgos atrocyanea* (Bas. *Pterospora atrocyanea* Métrod) is the type species of the genus *Tetrapyrgos*, established by Horak (1987) to replace the invalid *Pterospora* Métrod (1949) (non *Pterospora* Nuttall 1818, Pyrolaceae). The holotype specimen was collected in a government park in Antananarivo, Madagascar, and comprehensively described in the protologue (Métrod 1949). The holotype, preserved in alcohol, was studied by Horak (1968) who provided more details on micromorphology. As noted by Honan et al (2015), the documentation of tetrahedral basidiospores, *Rameales*-type pileipellis and stipitipellis, diverticulate cheilocystidia with apical knobs, bluish stains, and a stipe with white apex and bluish black base provided enough data to help circumscribe the genus *Tetrapyrgos*. However, the poorly preserved holotype and absence of recently collected material prohibited generation of molecular sequences to help distinguish the taxon from similar species, especially from *T. longicystidiata* A.H. Honan, Desjardin & T.J. Baroni, newly described from neotropical populations, from *T. subcinerea* (Berk. & Broome) E. Horak, described from Sri Lanka and widely distributed throughout south and southeast Asia, and from the genus *Campanella*. During recent fieldwork in Madagascar, co-authors Shay and Newman encountered several specimens of *Tetrapyrgos*, and one in particular (JES 216) was collected in a government park in Antananarivo, quite possibly the same park from which the holotype originated. We herein accept this specimen as epitype material for *T. atrocyanea*.

The morphology of JES 216 matches quite nicely that described in the protologue (Métrod 1949) and subsequent type study (Horak 1968), differing only slightly in basidiospore size. Métrod (1949) reported the basidiospores as "7–8 × 2.5–3.5 µm, ovoid with two lateral wings 2–2.5 µm long" (collectively 7–8 × 6.5–8.5 µm), while Horak (1968) noted the basidiospores as tetrahedral, 7–9 µm broad and long. Our material shows distinctly "winged", tetrahedral basidiospores 8–11.8 × 7–9 µm, i.e., up to several microns longer than reported for the holotype specimen.

Pairwise comparisons of aligned, overlapping ITS sequences of the epitype of *T. atrocyanea* with the top ten BLAST results indicate 99.9% similarity to the holotype specimen of *T. longicystidiata* (EF175544) from Puerto Rico, and 99.6% similarity to a specimen of *T. longicystidiata* from Bolivia (EF175533). In the protologue of *T. atrocyanea* (Métrod 1949; pg. 129, Fig. 87c) and Horak's type study (Horak 1968; pg. 528, Fig. e) they illustrated very long cheilocystidia (45–55 µm *sensu* Horak), the diagnostic feature of *T. longicystidiata* (Honan et al. 2015; pg. 109, Fig. 2d). These data suggest that the recently described new world taxon is a synonym of *T. atrocyanea*, described from Madagascar.

Campanella buettneri Henn., Bot. Jb. 22: 95. 1895. Figs. 3, 4a–d

Holotype – AFRICA, Togo, Bismarckburg, on wood, 1892, coll. by R. Büttner (B).

Epitype *hic designari* – AFRICA, Príncipe island, Bom Bom resort area, N1˚41.559', E7˚24.171', 20 April 2008, coll. by D.E Desjardin, *DED 8276* (MF075138–LSU, MF075136–ITS, SFSU)

MycoBank: MB 208007; Facesoffungi number: 03428

Description of epitype material:

Pileus 5–12 mm diam, auriform, asymmetrically convex to campanulate in profile, hemispherical to subchordate in face view, margin undulate, sometimes cleft; surface rugulose to pustulose, tessellate, dull, dry, white overall, drying white to pale cream, unstaining. Context thin, rubbery-pliant, white. Lamellae adnate to point of attachment, remote with many cross-lamellae, costate-reticulate, intervenose, relatively narrow, white, drying dark cream, unstaining. Stipe absent; with or without a poorly developed pseudostipe extending from the margin, usually in the cleft, or eccentrically from the pileus surface. Odor and taste not distinctive.

Basidiospores 8.6–10.2 × 6.0–7.2 µm [x_m = 9.7 ± 0.46 × 6.6 ± 0.34 µm, Q = 1.4–1.6, Q_m = 1.48 ± 0.04, n = 26, s = 1], broadly ellipsoid, rarely approaching subglobose, lacking bulges, smooth, hyaline, inamyloid, thin-walled. Basidia 28–32 × 7–8 µm, clavate, 4-spored. Basidioles clavate to subclavate, seldom subfusoid. Cheilocystidia not differentiated. Hymenial cystidia common on sides and edges of lamellae, 38–48 × 9–12 µm, fusoid to ventricose, hyaline, thin-walled

or with wall up to 0.5 µm thick (not metuloids). Pileipellis a well-developed *Rameales*-structure; hyphae 3–5 µm diam, cylindrical to irregular in outline, dendrohyphidia-like, densely diverticulate, hyaline, inamyloid, thin-walled, non-gelatinous; diverticula 2–12 × 1–2 µm, cylindrical to narrowly conical, mostly obtuse, hyaline, thin-walled. Pileus trama of loosely interwoven hyphae 2–4 (–5) µm diam, cylindrical, non-diverticulate, hyaline, inamyloid, embedded in a strongly gelatinous matrix. Lamellar trama regular, hyphae 2.5–5 µm diam, hyaline, inamyloid, gelatinous. Clamp connections common in all tissues.

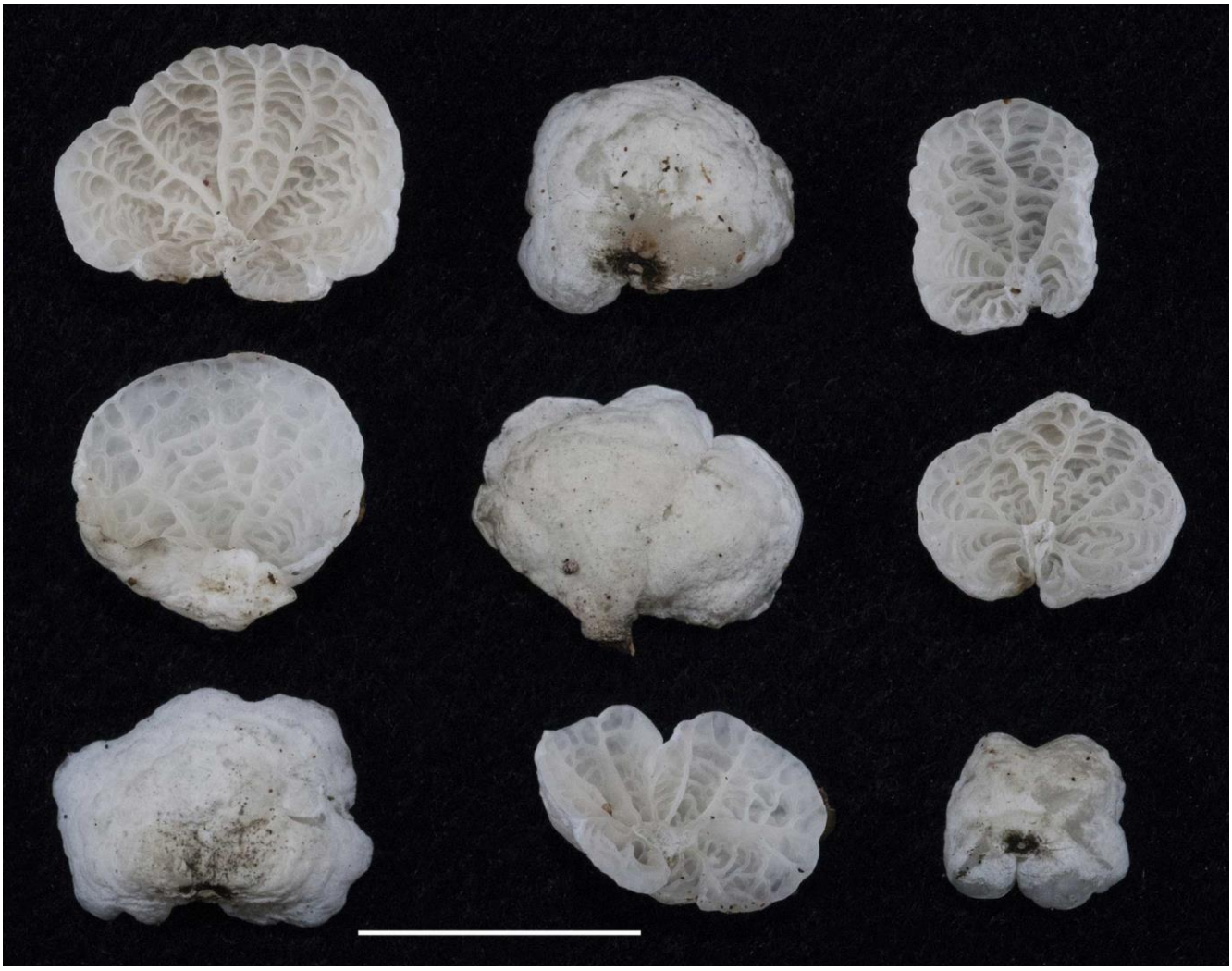

Fig. 3 – Basidiomes of *Campanella buettneri* (Epitype, DED 8276). Scale bar = 10 mm

Photograph by BA Perry

Habitat and known distribution – Gregarious on woody debris of undetermined dicotyledonous plants. Africa (Cameroon, Príncipe, São Tomé, Togo), Hawaii.

Additional material examined – São Tomé, Parque Nacional Obo, along trail from Bom Succeso to Lagoa Amelia, N0˚17.317', E6˚36.746', 28 April 2006 and 2 May 2006, coll. by D.E. Desjardin, material lost in transit. United States. Hawaiian Islands, Hawai`i, Lava Tree State Park near Leilani, N19˚28'08", W154˚54'10", 30 July 2005, coll. by Ed Lickey, TFB 12565 (TENN-F-60782).

Notes – *Campanella buettneri* is the type species of the genus *Campanella*, established by Hennings (1895) from material collected in Togo and Cameroon. We are unaware of recently collected material from these countries upon which the generic concept of *Campanella* can be refined, but we have collected material from the nearby island nation

of São Tomé and Príncipe that we recognize as representing this important species, and we herein accept it as epitype material for *C. buettneri*.

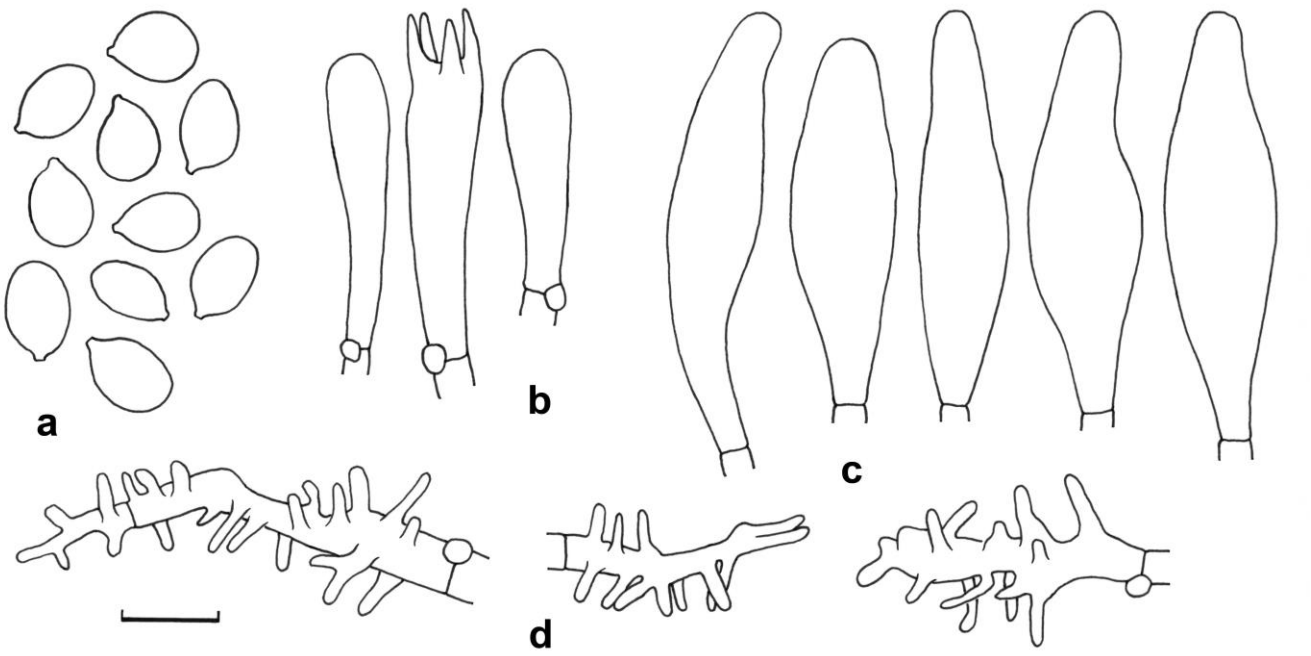

Fig. 4 – Micromorphological features of *Campanella buettneri* (Epitype, DED 8276). a. Basidiospores. b. Basidium and basidioles. c. Hymenial cystidia. d. Pileipellis hyphae. Scale bar = 10 µm. Illustration by DE Desjardin.

The Príncipe specimen matches the protologue (Hennings 1895) and differs only slightly from an analysis of the holotype specimen studied by Singer (1975). *Campanella buettneri* is characterized by small, rubbery-pliant basidiomes with a white, ear-shaped, tessellate-verrucose pileus 5–12 mm diam attached to the substrate by a small, eccentric to lateral pseudostipe, costate-reticulate, intervenose, white hymenophore, broadly ellipsoid basidiospores with mean 9.7 × 6.6 µm, fusoid-ventricose hymenial cystidia, a *Rameales*-type pileipellis, and growth on woody debris. The protologue reported the basidiospores as subglobose, 7–9 µm, while Singer (1975) reported those in the holotype specimen as ellipsoid, 8.7 × 6.3 µm; neither author mentioned lateral bulges on the basidiospores. The basidiospores of the Príncipe material are mostly broadly ellipsoid with only a few tending towards subglobose, but the range in variation encompasses the sizes reported by Hennings (1895) and Singer (1975); likewise, they lack lateral bulges. Additional noteworthy features of the species include the distinct fusoid-ventricose, non-refractive, thin- to firm-walled hymenial cystidia, strongly gelatinous pileus trama, a non-gelatinous pileipellis of irregular hyphae with relatively long, narrow diverticula, and white basidiomes that do not discolor greyish green.

Singer (1975) compared *C. buettneri* to *C. alba* (Berk. & M.A. Curtis) Singer, a species described from Cuba and rather widespread in the neotropics, indicating that the latter differed primarily in having basidiospores with lateral bulges. *Campanella alba* differs also in forming few, scattered hymenial cystidia that are narrowly cylindrical (2.7–7.5 µm) and subcapitate to capitate, whereas in *C. buettneri* the hymenial cystidia are fusoid-ventricose, 9–12 µm diam, and non-capitate. Pairwise comparisons of aligned, overlapping ITS sequences of *C. buettneri* with the top ten BLAST results indicate closest similarity (99.5%) to a sequence determined as *C. alba* (DQ449943) obtained from a specimen collected on Hawai`i island, USA. Examination of the latter specimen (TENN-F-60782) indicated morphology nearly identical to that of the epitype specimen designated herein (albeit with fewer hymenial cystidia), and we recognize the Hawaiian material as representing *C. buettneri*, not *C. alba*.

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