RESEARCH ARTICLE

Outstanding Pinkish Brown-Spored Neotropical Boletes: Austroboletus subflavidus and Fistulinella gloeocarpa (Boletaceae, Boletales) from the Dominican Republic

Matteo Gelardi, Claudio Angelini, Federica Costanzo, Enrico Ercole, Beatriz Ortiz-Santana and Alfredo Vizzini

ABSTRACT
The occurrence of Austroboletus subflavidus and Fistulinella gloeocarpa is documented from the Dominican Republic. The latter species is reported for the first time outside its original locality in Martinique, extending the geographic range for this uncommon pinkish-spored bolete. A detailed morphological description is provided for each species and accompanied by color pictures of fresh basidiomes in habitat and line drawings of the main anatomical features. Both species represent independent lineages within their respective genera based on phylogenetic inference. In addition, A. subflavidus clusters in a sister lineage to the core Austroboletus clade (Austroboletus clade I) here named as Austroboletus clade II. In order to confirm the accuracy of species identification, their identity and relationships were subjected to multilocus phylogenetic analyses of three gene markers (ITS, nrLSU, RPB2) including genetic material already available in public databases. Austroboletus subflavidus is a widely distributed species in North and Central America, whereas F. gloeocarpa is apparently highly localized and seems to appear sparingly in the Dominican Republic, Martinique, and southern Florida. Comparisons with morphologically similar and molecularly inferred allied species are also presented and discussed.

1. Introduction
With the recent advancement of molecular techniques applied to the study of boletoid mushrooms and related groups (Boletaceae, Boletales), several different generic and infrageneric lineages have been extensively investigated, revealing an extraordinary diversity mainly distributed across temperate, subtropical, and tropical environments of both hemispheres [1–8]. Yet, the increasing number of genera in the Boletales has barely been investigated with a molecular approach, thus determining largely unresolved phylogenetic relationships, unclear taxonomic limits, and often revealing a polyphyletic nature in their original circumscription, as in the case of the pinkish brown-spored Fistulinella Henn. and Austroboletus (Corner) Wolfe.

E.J.H. Corner first introduced Austroboletus, typified by Porphyrellus dicytous Boedijn, as a subgenus of his broadly conceived Boletus Fr. s. l. to accommodate a number of Malaysian boletes with ornamented basidiospores [9]. A few years later, E. Horak [10] stated that "according to our personal experience with tropical species of Strobiliomycetaceae at least Heimiella and subgen. Austroboletus have to be considered as good and independent genera within the taxonomic framework of the boletes". C.B. Wolfe and R.H. Petersen critically reevaluated the infrageneric limits of Porphyrellus E.-J. Gilbert s. l. and Boletus subgen. Austroboletus [11] and shortly after Wolfe [12] upgraded Austroboletus to genus rank, providing further insights into the taxonomy and a comprehensive revision of several type specimens. The recognition of Austroboletus at the generic rank was subsequently disputed by Corner [13] but accepted and integrated with additional taxa and new combinations by Pegler and Young [14], Singer [15,16], and by Horak [17,18], Watling and Gregory [19], and Singer et al. [20] based on fungal material yielded in Australasia and Latin America. Austroboletus currently comprises some 36 species [21] and incorporates taxa assigned by Singer [22] to Porphyrellus sect. Graciles Singer and sect. Tristes Singer and successively placed by Smith and Thiers.
Colombia \[31\], India \[48,49\] and Australia \[26\], this Austroboletus and molecularly-based novel species assigned to Despite the increasing number of morphologically with other lineages in the Boletaceae \[7\].

As they cluster in an well-delimited grouping with subfamily Austroboletoideae G. Wu & Zhu L. Yang, legumes \[8,9,12,14,16\]. Dipterocarpaceae, Myrtaceae, and caesalpinoid plant families including Fagaceae, Pinaceae, ectomycorrhizal (ECM) association with several giocarpic (secondary angiocarpic) ontogenesis and velangiocarpic (primary angiocarpy), or pseudoan-

edly reticulate-alveolate, lacerate or lacunose stipe, brownish pink tubular hymenophore at maturity, cream becoming flesh-pink to vinaceous pink or brownish pink tubular hymenophore, slim stipe with a smooth, rarely reticulate but not alveolate-lacunose surface, unchanging tissues, vinaceous pink to reddish brown or rust brown to cocoa brown spore print, narrowly elongate fusoid, inamyloid to dextrinoid, smooth basidiospores, trichoderm to ixotrichoderm or ixocutis pileipellis, strongly gelatinized bilateral-divergent hymenophoral trama of the “Boletus-type”, suspected gymnocarpic ontogenesis in some species but probably also velangiocarpic (primary angio-

arity) in others and presumably but not proved ECM association with members of the Polygonaceae, Sapotaceae, Myrtaceae, Euphorbiaceae, Fagaceae, Nothofagaceae, and caesalpinoid legumes in mesophytic and hygrophytic forests \([11,14,16,20,21,30,32,50,52–58]\) this study). The biogeographic distribution of Fistulinella is more or less overlapping that of Austroboletus, the majority of species being distributed in the pantropical belt with only a few extending to temperate regions of both northern and southern hemispheres \[16,32\]. Despite the long-

standing of Fistulinella, an unanimous taxonomic interpretation of the genus has never been reached \[6,27,56\]. From the phylogenetic perspective, Fistulinella is inferred to be related to Austroboletus, Maciophilus, Veloporphyrellus, and apparently Carolinigaster \[5,7,8,37–39,43,45–47\] and it seems to occupy a sister position to the remainder of the Austroboletoidae \[5,7,39,41,43,45,47\]. On the other hand, preliminary molecular analyses suggested this genus to be polyphyletic \[31,37\] and accordingly an inclusive revision complemented by further sampling from different geographic regions aiming at a better understanding of its generic boundaries would be urgently needed, especially in relation to morphologically very close smooth-spored genera such as Maciophilus and Ixechinus R. Heim ex R. Heim. Moreover Vasco-Palacios et al. \[31\], and Magnago et al. \[42\] have stressed that American species belonging in Fistulinella cluster in a statistically strongly supported separate clade with respect to those described from Australia and New Zealand, but

**Fistulinella**, typified by *F. staudtii* Henn., was first recognized by the German mycologist P. Hennings at the beginning of the twentieth century based on material recorded in Cameroon, central Africa \[50\]. The genus includes the species assigned by Singer \[51\] to Porphyrellus sect. Pseudotylopilus subsect. Viscidini Singer and encompasses at present more than 20 species worldwide \[21\]. Fistulinella is character-

ized by stipitate-pileate to occasionally sequestrate fruiting bodies having relatively small size, slender and gaunt habit, velate, or evelate, usually viscid to strongly glutinous pileus and stipe surfaces, pileus sometimes scrobiculate, initially whitish becoming pinkish to vinaceous pink or brownish pink tubular hymenophore, slim stipe with a smooth, rarely reticulate but not alveolate-lacunose surface, unchanging tissues, vinaceous pink to reddish brown or rust brown to cocoa brown spore print, narrowly elongate fusoid, inamyloid to dextrinoid, smooth basidiospores, trichoderm to ixotrichoderm or ixocutis pileipellis, strongly gelatinized bilateral-divergent hymenophoral trama of the “Boletus-type”, suspected gymnocarpic ontogenesis in some species but probably also velangiocarpic (primary angio-

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it is not until molecular analyses are carried out on the
generic type, the African taxon *F. staudeii*, that a
taxonomic and geographic delimitation of *Fistulinella*
s. str. lineage will be definitely clarified.

In order to reconstruct the phylogeny of
*Austroboletus* and *Fistulinella*, nucleotide sequences of
three regions, viz., the nuclear ribosomal internal
transcribed spacer (ITS) region, large subunit nuclear
ribosomal RNA gene (nrLSU) and DNA-directed RNA polymerase II subunit gene (RPB2),
were generated in this study from samples of *A.
subflavidus* and *F. gloeocarpa* recently recorded in
the Dominican Republic (Greater Antilles). Given
the limited number of mycological studies under-
taken in the island, it is not at all surprising to find
out distinctive bolete genera and species that were
previously scarcely documented or com-
pletely overlooked.

2. Materials and methods

2.1. Collection site and sampling

Specimens were collected in Jarabacoa, La
Vega Province and Sosúa, Puerto Plata Province,
Dominican Republic, and are deposited in the
Herbarium of Jardin Botánico Nacional de Santo
Domingo, Dr. Rafael Ma. Moscoso, Dominican
Republic (JBSD) (acronym from Thiers [59]), while
"ANGE" and "MG" refer to the personal herbarium
of Claudio Angelini and Matteo Gelardi, respec-
tively. Herbarium numbers are cited for all collec-
tions from which morphological features were
examined. Author citations follow the Index
Fungorum, Authors of Fungal Names (www.index-
fungorum.org/authorsoffungalnames.htm).

Geographic distribution and morphological features
of the studied species have also been checked on
MyCoPortal (https://mycoportal.org) and the NYBG
Boletineae project (https://sweetgum.nybg.org/sci-
ence/projects/boletineae/), respectively.

2.2. Morphological studies

Macroscopic descriptions, macro-chemical reactions
(30% NH₄OH, 30% KOH) and ecological inform-
ation, such as habitat notations, time of fruiting, and
associated plant communities accompanied the
detailed field notes of the fresh basidiomes. In the
field, latitude, longitude, and elevation were deter-
dined with a Global Positioning System (GPS)
receiver. Color terms in capital letters (e.g., White,
Plate LIII) are from Ridgway [60]. Photographs of
collections were taken in the natural habitat using a
Nikon Coolpix 8400 camera. Microscopic anatom-
ical features were observed and recorded from
revived dried material; sections were rehydrated
either in water, 5% KOH or in anionic solution
saturated with Congo red. All anatomical structures
were measured from preparations in anionic Congo
red. Colors and pigments were described after
examination in water and 5% KOH. Measurements
were made at 1000× using a calibrated ocular micrometer (Nikon Eclipse E200 optical light micro-
scope). Basidiospores were measured directly from
the hymenophore of mature basidiomes, dimensions
are given as (minimum) average ± standard devi-
ation (maximum), Q = length/width ratio with the extreme values in parentheses, Q_m = average quo-
tient (length/width ratio) ± standard deviation and
average spore volume was approximated as a rota-
tion ellipsoid \[ V = (\pi L W^2)/6 \pm SD \]. The notation
[n/m/p] indicates that measurements were made on
"n" randomly selected basidiospores from "m" basi-
diomes of "p" collections. The width of each basi-
dium was measured at the widest part, and the length
was measured from the apex (sterigmata excluded)
to the basal septum. Radial and/or vertical sections
of the pileipellis were taken midway between the
center and margin of the pileus. Sections of the stip-
tipellis were taken from the middle part along the
longitudinal axis of the stipe. Metachromatic, cyano-
philic, and iodine reactions were tested by staining
the basidiospores in Brilliant Cresyl blue, Cotton
blue, and Melzer’s reagent, respectively. Line draw-
ings of microstructures were traced in free hand
based on digital photomicrographs of rehy-
drated material.

2.3. DNA extraction, PCR amplification, and
DNA sequencing

DNA extraction and PCR amplification were per-
formed from dried basidiomata (Table 1) as
described by Vizzini et al. [75]. Primers ITS1F and
ITS4 [76,77] were used for the ITS region; primers
LR0R and LR5 [78,79] were used for the nrLSU.
Amplifications of the RPB2 gene were attempted
using the primers bRPB2-6F2, bRPB2-7.1R2, and
bRPB2-7R2 [80,81]. The PCR products were puri-
ified with the Wizard SV Gel and PCR Clean-Up
System (Promega, Madison, WI) following manufac-
turer’s instructions and positive reactions sequenced
forward and reverse by MACROGEN Inc. (Seoul,
Republic of Korea).

2.4. Sequence alignment, data set assembly, and
phylogenetic analyses

The sequences obtained in this study were checked
and assembled using Geneious v. 11.1.4 [82] and
compared to those available in GenBank by using
the Blastn algorithm [83]. Chromatograms were
Table 1. Details of specimens used in the phylogenetic analyses.

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(continued)
examined and manually edited for accuracy. Newly acquired sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and samples with accession numbers are listed in bold typeface in Table 1. Homologous sequences from vouchered specimens and from environmental samples were selected and retrieved from GenBank (see Table 1). Newly obtained sequences are in bold.

Alignments were generated for the ITS, nrLSU, and RPB2 datasets with MAFFT [84] with default conditions for gap openings and gap extension penalties. Alignments were then manually adjusted and concatenated using Geneious v. 11.1.4 [82]. We estimated the best fit substitution model for each single alignment using the Bayesian information criterion (BIC) with jModelTest 2 [85] and therefore selected the TIM1 + G, TIM2 + G, and K80 + G models for nrLSU, ITS, and RPB2, respectively. The ITS dataset was not partitioned. A combined nrLSU/ITS/RPB2 analyses focused on the Austroboletoideae as circumscribed by Wu et al. [7,8] was performed. Sequences of Austroboletus betulaceus [3,86] were not included in the analyses because the species was recently inferred to belong in Aureoboletus Pouzar within the subfamily Xerocomoideae [5]. Bothia and Salioccasus sequences were used as outgroup according to Wu et al. [7,8] and Magnago et al. [42]. Phylogenetic trees were constructed with Bayesian inference (BI) and Maximum likelihood (ML) criteria. The partitioned BI was performed with MrBayes v. 3.2.7a [87] with one cold and three incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 M generations, under the selected evolutionary models for each unlinked partition. Two simultaneous runs were performed independently. Trees were sampled every 1000 generations, resulting in sampling of 10001 trees per single run with the first 2500 trees (25%) discarded as burn-in. For the remaining trees of the two independent runs, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPPs). Partitioned ML analyses were performed using RAxML v. 7.3.2 [88] with 1000 bootstrap replicates [89] and the GTR+GAMMA model of sequence evolution. Support values from bootstrapping runs (MLB) were mapped on the best ML tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway [90]. BPP values ≥0.95 and MLB values ≥70%, are reported in the resulting tree (Figure 1). Lower values are exceptionally represented inside parentheses. Branch lengths were estimated as mean values over the sampled trees. Pairwise percent identity values (P %IV) of the ITS sequences were calculated using Geneious v. 11.1.4 [82]. Alignments and phylogenetic trees are available at TreeBASE (www.treebase.org) under ID 26454.

3. Results

3.1. Molecular analyses

Both Bayesian and Maximum Likelihood analyses produced comparable topologies and therefore only Bayesian trees with BPP and MLB values are shown (Figure 1). The nrLSU dataset comprised 92 accessions and 974 characters. The ITS dataset included 76 taxa and 1397 characters. The RPB2 dataset is composed of 39 taxa and 527 characters. The combined dataset comprised 122 specimens (Table 1). The genera Austroboletus and Fistulinella, as currently morphologically circumscribed, are polyphyletic, as well as Veloporphyrellus (Figure 1). Two major strongly supported sister clades were recognized in Austroboletus, herein named as I (BPP = 1; MLB = 93%), including the type species A. dictyotus, and II (BPP = 1; MLB = 95%). Austroboletus festivus, A. gracilis, and A. rio-negrensis are independent evolutionary lineages outside Austroboletus. The sequences of A. subflavidus form a separate clade (BPP = 1; MLB = 99%) within major clade II. P%IV of the ITS sequences of the A. subflavidus clade is 97.3.

Most Fistulinella sequences cluster in a clade strongly supported only by the Bayesian analyses (BPP = 1; MLB = 65%) also including F. gloeocarpa. Fistulinella prunicolor and F. viscida fall outside the Fistulinella clade. The two Fistulinella gloeocarpa collections show a P%IV of 99.4.

3.2. Taxonomy


MYCOBANK MB 118437
≡*Boletus subflavidus* (Murrill) Murrill, Mycologia 30 (5): 525. 1938

Holotype: USA, Florida, Gainesville, under *Pinus* sp., 14 Aug 1937, W.A. Murrill, 15862 (FLAS); neo-
type designated by C.B. Wolfe [12]: USA, Florida, Gainesville, 11 Jul 1938, E. West, Arnold and W.A. Murrill (NY, isoncotype: FH); authentic material also preserved in NY and FLAS [91].

Basidiomes small. *Pileus* (1.4) 2.1–5.0 (5.5) cm broad, at first hemispherical then persistently convex to nearly planate, not depressed at center, regularly or rarely unevenly shaped by shallow depressions, moderately fleshy, firm at the beginning but progressively softer with age; margin obtuse, steady to faintly wavy-lobed, slightly involute then curved downwards, sterile and not or only a little extending beyond the tubes (up to 1 mm); surface matt, dry, very finely tomentose, soon disrupted and appearing typically areolate with age and showing the whitish (White, Pl. LIII) context beneath, rarely not cracked; cuticle patches color ranging from whitish, ivory, beige or pale cream yellowish (White, Pl. LIII; Maize Yellow, Martius Yellow, Pl. IV; Marguerite Yellow, Pl. XXX; Naphtalene Yellow, Straw Yellow, Pl. XVI) to ochraceous or pale ochraceous-olive (Deep Olive-Buff, Dark Olive-Buff, Pl. XL;
Primuline Yellow, Olive Lake, Buffy Citrine, Pl. XVI; Ecru Olive, Light Yellowish Olive, Isabella Color, Buffy Olive, Pl. XXX); not staining on handling or when injured; subcuticular layer white (White, Pl. LIII). Tubes at first thin then increasingly broader, initially shorter or as long as but later longer than the thickness of pileus context (up to 1.4 cm long), adnate at first but soon deeply depressed around the stipe apex, whitish (White, Pl. LIII) at first to pale flesh-pink (Flesh-Pink, Venetian Pink, Pl. XIII; Pale Salmon Color, Pl. XIV; Pale Purplish Vinaceous, Pale Grayish Vinaceous, Pl. XXXIX), then pinkish lilac (Pale Lavander Violet, Pale Mauve, Mauvette, Light Mauve, Pl. XXV; Light Pinkish Lilac, Pl. XXXVII; Pale Brownish Vinaceous, Pl. XXXIX) and finally brownish pink to dirty brownish (Sorghum Brown, Hay's Brown, Light Seal Brown, Pl. XXXIX), unchangeable when cut. Pores initially forming a flat surface, later convex to ascendant, at first small then gradually wider (up to 1 mm in diam.), simple, roundish to barely angular at maturity, concolorous with or slightly paler than tubes and very slowly and faintly darkening (Purplish Vinaceous, Livid Brown, Pl. XXXIX) on bruising or when injured, occasionally beaded by scattered watery droplets. Stipe (2.9) 4.5–7.5 (10.2) cm.

Figure 2. Austroboletus subflavidus basidiomes in habitat. (a) JBSD130773 (ANGE1145); (b–d) close up of the stipe, context, and pileus, respectively (b, d: JBSD130774, ANGE1146; c: JBSD130771, ANGE108); (e) JBSD130772 (ANGE388); (f) JBSD130771 (ANGE108). Scale bars: 1 cm. Photos by C. Angelini.
× (0.4) 0.6–1.8 (2.0) cm, constantly longer than pileus diameter, central to slightly off-center, solid, firm, dry but decidedly viscid with moist weather, straight or curved, cylindrical to more frequently gradually swollen toward the base, ending with a short taproot at the very base, apparently evelate; surface prominently reticulate to deeply reticulate-alveolate throughout, reticulate pattern consisting of longitudinally stretched, waxy anastomosing ribs, increasingly coarser and more prominent to distinctly folded toward the base; whitish (White, Pl. LIII) to ivory or beige (Maize Yellow, Pl. IV; Marguerite Yellow, Pl. XXX) in the upper three fourth, pale cream yellowish to ochraceous (Martius Yellow, Pl. IV; Naphtalene Yellow, Straw Yellow, Pl. XVI; Primuline Yellow, Pl. XVI) downwards, usually with pale brown (Chamois, Pl. XXX; Sudan Brown, Pl. III) spots or shades at the stipe base; whitish (White, Pl. LIII) to ivory or beige (Maize Yellow, Pl. IV; Marguerite Yellow, Pl. XXX) in the upper three fourth, pale cream yellowish to ochraceous (Martius Yellow, Pl. IV; Naphtalene Yellow, Straw Yellow, Pl. XVI; Primuline Yellow, Pl. XVI) downwards, usually with pale brown (Chamois, Pl. XXX; Sudan Brown, Pl. III) spots or shades at the stipe base; reticulum concolorous to pale cream yellowish (Martius Yellow, Pl. IV; Naphtalene Yellow, Straw Yellow, Pl. XVI), unchangeable when pressed; basal mycelium white (White, Pl. LIII). Context firm when young, later soft textured and eventually flabby in the pileus (up to 2.2 cm thick in the central zone), a little more fibrous in the stipe, white (White, Pl. LIII) throughout, usually with pale brown (Chamois, Pl. XXX; Sudan Brown, Pl. III) spots or shades at the stipe base; unchangeable when exposed to air; sub-hymenophoral layer white (White, Pl. LIII); excipitate pileus beige to pale olive brown (Maize Yellow, Pl. IV; Marguerite Yellow, Pl. XXX; Dark Olive-Buff, Pl. XL), hymenophore dull brown (Dull Brown, Pl. XXX), stipe and context beige (Maize Yellow, Pl. IV; Marguerite Yellow, Pl. XXX). Odor indistinct to faintly fruity. Taste bitter. Spore print not obtained. Macrochemical spot-test reactions: 30% KOH: none; 25% NH₄OH: pinkish on pileus, none on context.

Basidiospores [122/7/4] (13.1) 15.9 ± 1.15 (19.5) × (5.5) 7.0 ± 0.58 (8.7) μm, Q = (1.76) 1.87–2.61 (2.68), Qₚ = 2.26 ± 0.16, V = 416 ± 89 μm³ (including ornamentation), inequilateral, ellipsoid-fusiform, ellipsoid to broadly ellipsoid in side view, broadly ellipsoid to amygdaliform in face view, distinctly verrucose in central part by disruption of the outer wall, minutely pitted or furrowed to form irregular isolated, short rounded-tuberculate warts, or sinuous confluent meandering ridges 0.1–0.7 μm high, becoming progressively less pronounced toward both the apex and the distal end which appear minutely perforate-punctate or porose to nearly smooth, apex rounded, with a short apiculus and usually with a less ornamented suprahilar

Figure 3. Austroboletus subflavidus. Micromorphological features; (a) basidiospores; (b) cheilo- and pleurocystidia; (c) caulocystidia; (d) basidia; (e) elements of the pileipellis. Scale bars: 10 μm (a–d); 20 μm (e). Drawings by F. Costanzo.
applanation or shallow depression, often with a shallow abaxial depression close to the distal end and with an adaxial swelling, moderately thick-walled (0.5–1.0 μm), honey yellow colored in water, and 5% KOH, having one, less frequently two or three large oil droplets when mature, rarely pluriguttulate, inamyloid to faintly dextrinoid, strongly cyanophilic, and with a weak metachromatic reaction. Basidia 27–49 (51) × 12–19 μm (n = 18), sub-clavate to clavate or broadly clavate, moderately thick-walled (0.3–0.8 μm), predominantly 4-spored but also 1-, 2-, or 3-spored, usually bearing relatively short sterigmata (2–5 μm) (sterigmata up to 6 μm long in 1-spored basidia), hyaline to pale yellowish and seldom containing scattered straw-yellow oil guttules in water and 5% KOH, bright yellow (inamyloid) in Melzer’s, without basal clamps; basidioles faintly clavate to clavate, similar in size to basidia. Cheilocystidia (33) 35–65 (70) × 7–10 (12) μm (n = 12), uncommon, moderately slender, projecting straight to sometimes flexuous, irregularly cylindrical or cylindrical fusiform to fusiform with a narrow and long neck, sometimes mucronate, less frequently ventricose fusiform, with rounded to subacute tip, smooth, moderately thick-walled (0.5–0.8 μm), hyaline to pale yellowish in water and 5% KOH, bright yellow (inamyloid) in Melzer’s, without epiparietal encrustations. Pleurocystidia (36) 43–69 (73) × 8–12 μm (n = 9), infrequent, size, shape, color, and chemical reactions similar to cheilocystidia, occasionally lageniform, subclavate, mucronate to subcapitate. Pseudocystidia not recorded. Pileipellis a trichoderm consisting of strongly interwoven, elongated, frequently branched, filamentous and sinuous to cylindrical hyphae not to moderately embedded in gelatinous matter; terminal elements 27–100 × 4–15 μm, long and slender, filamentous and sinuous or short cylindrical to cystidioid, apex rounded-obtuse to more rarely pointed, thick-walled (up to 1.3 μm), hyaline to pale straw yellow in water and 5% KOH, golden yellow (inamyloid) in Melzer’s, smooth to occasionally ornamented by a very subtle granular epiparietal encrustation; subterminal elements similar in shape, size, and color to terminal elements. Stipitipellis a layer of slender, parallel to loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae, 2–11 μm wide, hyaline to very pale yellowish in water and 5% KOH; the stipe apex covered by a layer 300–400 μm thick of strongly entangled filamentous and sinuous, frequently branched hyphae 2–6 μm broad, having a wall up to 0.3 μm thick, heavily embedded in gelatinous matter, giving rise in the outermost part to a well-developed caulohymenial layer consisting of caulobasioides, projecting caulocystidia similar in shape, size, color and chemical reactions to hymenial cystidia, (50) 53–57 × (8) 10–14 μm (n = 5), having a wall up to 0.8 μm thick and very sparse caulobasioida mostly 1-, 2-, and 3-spored, 44–51 × 10–14 μm, sterigmata up to 6 μm long (n = 3). Lateral stipe stratum under the caulohymenium usually absent or not differentiated from the underlying layer but occasionally present, of the “boletoid type”, 30–40 μm thick and consisting of divergent, inclined and running toward the external surface, loosely intermingled and branched hyphae remaining separate and heavily embedded in a gelatinous substance. Stipe trama composed of densely arranged, subparallel to moderately interwoven, filamentous, smooth, inamyloid hyphae, 3–16 μm broad. Hymenophoral trama bilateral divergent of the “Boletus-type”, with slightly to strongly divergent, recurved-arcuate and loosely arranged, not-branched, distantly septate and generally not restricted at septa, gelatinous hyphae (lateral strata hyphae in transversal section not touching each other, (3) 4–8 (9) μm apart, 3–10 μm broad), hyaline to very pale yellowish in water and 5% KOH, inamyloid in Melzer’s; lateral strata (20) 30–40 (50) μm thick, mediostratum (15) 20–30 (40) μm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 3–8 μm broad, more frequently septate; in Congo Red the mediostratum is darker than the lateral strata. Oleipherous hyphae scattered although more frequently observed in the hymenium and basal stipe trama, golden yellow in 5% KOH and Melzer’s. Clamp connections absent in all tissues. Ontogenetic development probably gymnocarpic.

Edibility unknown.

Ecology and phenology: solitary to scattered or gregarious, growing on soil among litter in association with Pinus occidentalis in the Dominican Republic. Elsewhere associated with other pine trees (P. palustris, P. caribaea, etc.) and oaks (Quercus marilandica, Q. minima, Q. laurifolia, Q. virginiana, Q. oleoides, Q. humboldtii, etc.). Apparently uncommon at least in the Dominican Republic, fairly common to infrequent or occasional elsewhere. June to January.

Known distribution: eastern North America, eastern, and south-eastern USA (New Jersey south to Florida and west to Texas) down into the Gulf coastal plain and Mexico, Belize and Costa Rica in mainland Central America south to Colombia in northern South America, in the Greater Antilles Islands of the Caribbean reported from the Dominican Republic.

Examined material: DOMINICAN REPUBLIC, La Vega Province, Jarabacoa, Buena Vista, 19°11’09.3"N 70°35’16.9”W, 660 m, 22 Dec 2013, a single mature specimen, under P. occidentalis, C.
Angelini (JBSD130771, ANGE108, and MG775); same loc., 06 Dec 2014, a single middle-aged specimen, C. Angelini (JBSD130772, ANGE388, and MG776); same loc., Golf Club, 19°11′12.5″N 70°35′25.5″W, 800 m, 03 Jan 2020, several specimens in all developmental stages, C. Angelini (JBSD130773 and ANGE1145); same loc., 03 Jan 2020, three specimens two of which mature and the other one a primordium, C. Angelini (JBSD130774 and ANGE1146).

**Comments:** Originally described from northern Florida as a member of *Tylopilus* by Murrill [92], the species was then recombined in *Boletellus* Murrill by Snell [93] and subsequently transferred to *Porphyrellus* by Singer [94]. Some decades later Wolfe [12] placed it in *Austroboletus* where it is currently retained based on morphological and molecular inference.

*Austroboletus subflavidus* is readily distinguished among congenic species based on the small to medium-sized basidiomes (pileus up to 11 cm diam. and stipe up to 14.5 cm long and 5 cm wide), pileus surface dry and becoming rimose-areolate with age, whitish beige or pale cream yellowish to ochraceous olive, occasionally with a pale pinkish tinge, pinkish hymenophore, slender, deeply reticulate-alveolate, whitish beige to yellowish stipe usually showing brownish shades or patches at the base, white context and basal mycelium, unchanging tissues on brownish shades or patches at the base, white con-whitish beige to yellowish stipe usually showing a delicately reticulate pattern, smaller basidiomes (11.2–14 × 6.3–7 μm, Qm = 1.8) with an alveolate-reticulate equatorial ornamentation, smaller basidia (28–35 × 10–14 μm) and the occurrence on the other side of the Pacific Ocean in wet sclerophyll forests under Myrtaceae and Casuarinaceae in New South Wales, Australia [26].

Finally, *A. appendiculatus* differs from *A. subflavidus* by the pale brown pileus surface, yolk yellow or golden yellow to pale orange stipe, mild taste, slightly shorter basidiospores (14.2–16.5 × 7.3–9.1 μm, Qm = 1.83), clavate to subclavate or subventricose, larger caulocystidia (40–70 × 11–20 μm) and the occurrence under *Shorea robusta* (Dipterocarpaceae) in India [49].

**Fistulinella gloeocarpa** Pegler, Kew Bulletin Additional Series 9: 591. 1983 Figures 4 and 5. MYCOBANK MB 124413

**Holotype:** Lesser Antilles, Martinique, Terreille, on soil in secondary mesophitic forest, 200 m, 10 Oct 1975, J.P. Fiard, 611 A, B (K)

**Basidiomes** small. *Pileus* (1.5) 2.0–5.0 (5.5) cm broad, at first hemispherical then persistently convex and finally broadly pulvinate-flattened, sometimes slightly depressed at center, regularly to hardly unevenly shaped by shallow depressions, moderately fleshy, firm at the beginning but progressively softer with age, flabby in old basidiomes; margin obtuse,
steady to faintly wavy-lobed, initially slightly involute soon curved downwards and finally nearly completely plane, not or only a little extending beyond the tubes; surface matt, in the early developmental stages with an innermost gelatinous pellicle underly-
ing a dry, very finely rugulose-granulose outermost layer, later progressively smooth and glabrous, sometimes hammered to delicately wrinkled or coarsely ridged-reticulate (scrobiculate) due to the coagulation of the gelatinous layer, always strongly glutinous with age, irrespective of the weather conditions, not cracked; cuticle decidedly variable in color depending on the weather, ranging from pure white, whitish or pale grayish white to pale brownish gray (White, Pl. LIII; Pale Drab-Gray, Light Grayish Olive, Light Drab, Drab, Pl. XLVI) when rainy but typically darker, mouse gray or slate gray to brown, dark brown or blackish brown (Mouse Gray, Deep Mouse Gray, Iron Gray, Pl. LI; Sudan Brown, Antique Brown, Argus Brown, Raw Umber, Pl. III; Buckthorn Brown, Dresden Brown, Mummy Brown, Pl. XV; Dark Mouse Gray, Blackish Mouse Gray, Plate LI) when dry, in young specimens always with a narrow white (White, Pl. LIII) marginal rim; not staining on handling or when injured; subcuticular layer white (White, Pl. LIII) to mouse gray or slate gray (Mouse Gray, Deep Mouse Gray, Iron Gray, Plate LI). Tubes at first thin then increas-
ingly broader and decidedly longer than the thickness of the pileus context (up to 1.8 cm long), adnexed to deeply depressed around the stipe apex to nearly free, whitish (White, Pl. LIII) at first then
whitish pink to pale flesh-pink, light pinkish lilac (Light Buff, Pl. XV; Seashell Pink, Pale Salmon Color, Pl. XIV; Flesh-Pink, Chatenay Pink, Pl. XIII; Pale Purplish Vinaceous, Pale Grayish Vinaceous, Pl. XXXIX; Light Pinkish Lilac, Pl. XXXVII; Brownish Vinaceous, Deep Brownish Vinaceous, Pl. XXXIX) at maturity and further darkening up to cocoa brown (Sayan Brown; Wood Brown, Pl. XL) in old fruiting bodies, unchangeable when cut. **Pores** initially hidden by a thick, colorless, glutinous veil which soon disrupts revealing the fertile tissue underneath; at the beginning forming a flat surface, later slightly convex to ascendant, at first relatively small then gradually wider (up to 2 mm in diam.), simple, roundish to barely angular at maturity, colorless with the tubes and not staining on bruising or when injured. **Stipe** (3.0–5.0) 5.0–7.5 (8.0) × (0.4) 0.6–1.1 (1.3) cm, usually longer than or less frequently as long as the pileus diameter at maturity, central to slightly off-center, solid, firm, straight or curved, cylindrical to more frequently slightly swollen toward the base, ending with a short taproot at the very base; entirely enveloped by a thick, colorless, glutinous membrane which soon disrupts in velar remnants forming an ascending, persistent glutinous annulus located in the upper part of the stipe, eventually becoming cocoa brown (Sayan Brown; Wood Brown, Pl. XL) due to spore discharge; very finely pruinose to smooth and glabrous, devoid of reticulum; white (White, Pl. LIII) throughout but usually with cream yellowish, ochraceous yellow (Martius Yellow, Pl. IV; Naphtalene Yellow, Straw Yellow, Pl. XVI; Primuline Yellow, Pl. XVI) to bright flesh-pink (Light Coral Red, Pl. XIII; Salmon Color, Apricot Buff, Pl. XIV) spots or shades at the stipe base, unchangeable when pressed; basal mycelium white (White, Pl. LIII), rhizomorphs brownish (Mikado Brown, Pl. XXIX). **Context** firm when young, later soft textured and eventually flabby in the pileus (up to 0.7 cm thick in the central zone), a little more fibrous in the stipe, white (White, Pl. LIII) throughout but in young specimens with a mouse gray or slate gray (Mouse Gray, Deep Mouse Gray, Iron Gray, Plate LI) band just beneath the cuticle, with cream yellowish, ochraceous yellow (Martius Yellow, Pl. IV; Naphtalene Yellow, Straw Yellow, Pl. XVI; Primuline Yellow, Pl. XVI) to bright pinkish (Light Coral Red, Pl. XIII; Salmon Color, Apricot Buff, Pl. XIV) spots or shades at the stipe base; unchangeable when exposed to air; subhymenophoral layer white (White, Pl. LIII); exsiccate pileus dull grayish to brownish (Pale Drab-Gray, Light Grayish Olive, Light Drab, Drab, Pl. XLVI), hymenophore flesh-pink to cocoa brown

**Figure 5.** *Fistulinella gloeocarpa*. Micromorphological features; (a) basidiospores; (b) cheilocystidia; (c) pleurocystidia; (d) stipitpellis; (e) basidia; (f) elements of the pilepellis. Scale bars: 10 μm (a–c, e); 20 μm (d, f). Drawings by F. Costanzo.
(Sayal Brown; Wood Brown, Pl. XL), stipe and context whitish to beige (White, Pl. LIII; Maize Yellow, Pl. IV; Marguerite Yellow, Pl. XXX). Odor indistinct. Taste mild. Spore print cocoa brown (Sayal Brown; Wood Brown, Pl. XL). Macromorphological observations: 30% KOH: staining wine red everywhere; 25% NH₄OH: none.

Basidiospores [70/7/3] (10.3) 12.8 ± 1.00 (16.2) × (4.5) 5.3 ± 0.34 (6.4) μm, Q = (2.00) 2.03 – 2.75 (2.84), Qₐ = 2.40 ± 0.17, V = 193 ± 35 μm³, inequilateral, ellipsoid fusiform to fusiform in side view, ellipsoid to ellipsoid fusiform in face view, smooth, apex rounded, with a short apiculus, usually with a shallow suprahilar depression and a slightly pronounced adaxial swelling, moderately thin-walled (0.3 – 0.5 μm), straw yellow colored in water and 5% KOH, having one, less frequently two or three large oil droplets at mature, rarely pluri-guttulate, inamyloid, strongly cyanophilic and with an orthochromatic reaction. Basidia (21) 23 – 38 (40) × 10 – 14 μm (n = 20), subclavate to clavate, moderately thick-walled (0.3 – 0.8 μm), predominantly 4-spored but also 1-, 2-, or 3-spored, usually bearing relatively short sterigmata (2 – 5 μm) (sterigmata up to 8 μm long in 1-spored basidia), hyaline to pale yellowish and seldom containing scattered straw-yellow oil gullets in water and 5% KOH, bright yellow (inamyloid) in Melzer’s, without basal clamps; basidioles cylindrical-clavate, faintly clavate to clavate, similar in size to basidia. Cheilocystidia (37) 39 – 56 (60) × 5 – 9 μm (n = 13), common, moderately slender, projecting straight to sometimes flexuous, irregularly cylindrical or cylindrical fusiform to narrowly fusiform, with rounded to subacute, smooth, moderately thick-walled (0.5 – 1.0 μm), hyaline to pale yellowish in water and 5% KOH, bright yellow (inamyloid) in Melzer’s, without epiparietal encrustations. Pleurocystidia (32) 36 – 58 (65) × (6) 8 – 13 μm (n = 10), infrequent, color, and chemical reactions similar to but with a different shape, fusiform to ventricose fusiform or lageniform and broader than cheilocystidia. Pseudocystidia not recorded. Pileipellis an ixotrichoderm consisting of interwoven, elongated, frequently branched, filamentous and sinuous to cylindrical, disarticulating and easily detached hyphae heavily embedded in gelatinous matter; terminal elements 22 – 135 × (4) 5 – 22 μm, long and slender, filamentous and sinuous to large cylindrical or sausage-shaped, apex rounded-obtuse, thick-walled (up to 2 μm), hyaline to very pale yellowish in water and 5% KOH, golden yellow (inamyloid) in Melzer’s, smooth to sometimes ornamented by a very subtle granular epiparietal encrustation; subterminal elements similar in shape, size and color to terminal elements. Stipitipellis a layer of slender, parallel to loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae, 5 – 10 μm wide, hyaline to very pale yellowish in water and 5% KOH; the stipe apex covered by a layer 100 – 150 μm thick of strongly entangled filamentous and sinuous, frequently branched hyphae 2 – 5 μm broad, having a wall up to 0.3 μm thick, heavily embedded in gelatinous matter, giving rise in the outermost part to disrupted tufts of projecting parallel to subparallel and anticlinally arranged, septe hyphae; terminal elements short cylindrical to irregularly cylindrical or subclavate to peanut-shaped or acorn-shaped, 15 – 54 × 7 – 10 μm, occasionally filamentous and up to 80 × 4 μm, apex rounded-obtuse; caulohymenial elements not differentiated or nearly so, caulobasidia infrequent, mostly 1- and 2-spored, 35 – 45 × 7 – 10 μm, sterigmata up to 7 μm long (n = 6), caulocystidia not observed. Lateral stipe stratum absent. Stipe trama composed of confusedly and densely arranged, subparallel to moderately interwoven, filamentous, smooth, inamyloid hyphae, 4 – 22 μm broad. Hymenophoral trama bilateral divergent of the “Boletus-type”, with slightly to strongly divergent, recurved-arcuate and loosely arranged, not-branched, distantly septate and generally restricted at septa, gelatinous hyphae (lateral strata hyphae in transversal section not touching each other, (4) 5 – 12 (15) μm apart, 5 – 12 μm broad), hyaline to very pale yellowish in water and 5% KOH, inamyloid in Melzer’s; lateral strata (20) 30 – 80 (90) μm thick, mediostratum (10) 20 – 40 (50) μm thick, axially arranged, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 2 – 8 μm broad, more frequently septate; in Congo Red the mediostratum is darker than the lateral strata. Oleiphorous hyphae scattered although more frequently observed in the basal stipe trama, golden yellow to brownish in 5% KOH and Melzer’s. Clamp connections absent in all tissues. Ontogenetic development probably hemiangiocarpic (monovelangiocarpic) due to the presence of a thick, glutinous and colorless, universal veil enveloping the entire basidiomes.

Edibility unknown.

Ecology and phenology: gregarious, growing on limestone among litter in a seasonally dry and moist anthropized lowland mixed stand under a large array of neotropical broadleaved trees including Coccoloba diversifolia (Polygonaceae) and (in Martinique) perhaps also with Haematoxylum sp. (Caesalpinioideae), which represent its possible ECM host trees. See Parra et al. [107] for further details on lowland vegetation in the Dominican Republic. Apparently localized in the Dominican Republic. August to March.

Known distribution: to date only known from both the Lesser and Greater Antilles islands of the Caribbean (Martinique, the Bahamas and the
Dominican Republic) and in all probability in south-eastern USA (Florida) in tropical environment (see below).

Examined material: DOMINICAN REPUBLIC, Municipality of Sosúa, Puerto Plata Province, loc. cemetery, three km away from the seaside, 19°44′40″N 70°32′21″W, 100 m, 01 Dec 2017, several specimens in all developmental stages, C. Angelini (JBSD130769, ANGE969, and MG777); same loc., 02 Dec 2017, several specimens in all developmental stages, C. Angelini (JBSD130770, ANGE970, and MG778); same loc. 28 Mar 2020, a single mature specimen, C. Angelini (ANGE1147).

Comments: Macro-morphologically, anatomically, and ecologically, samples in this study almost perfectly match the description of Fistulinella gloeocarpa described by Pegler [55] from Martinique (Lesser Antilles) based on material mostly collected by J.P. Fiard and by Pegler. This species can be recognized on account of the following combination of characters: small basidiomes (pileus up to 5.5 cm diam.), pileus surface at first dry and rugulose-granuloose to progressively smooth, sometimes hammered to finely wrinkled-reticulate or scrobiculate and then strongly glutinous with age, ranging from whitish, grayish or grayish brown to dark brown or blackish brown, white to pinkish hymenophore covered by a thick, colorless and glutinous veil in early developmental stages, smooth, glutinous, white stipe usually showing yellowish ochraceous shades or patches at the base and with a persistent glutinous annulus at maturity, white context and basal mycelium, unchanging tissues on bruising or injury, mild taste, cocoa brown spore deposit, reddish staining reaction with KOH on all tissues, ellipsoid-fusiform, smooth, narrowly cylindrical basidiospores, ixotrichoderm pileipellis consisting of filamentous to cylindrical hyphae and the occurrence in low-elevation neotropical environments in alleged association with Coccoloba diversifolia (this plant was found at the collection sites in both Martinique and the Dominican Republic) and Haematoxyllum sp. ([55]; this study).

A considerable amount of specimens collected in the field in the Dominican Republic has given us the opportunity to recognize reliable discriminating features for separating F. gloeocarpa from a number of morphological lookalikes occurring in Central and northern South America, such as F. jamaicensis (Murrill) Singer, F. venezuelae (Singer & Digiilio) Singer, F. mexicana Guzmán, F. campinaranae Singer, F. cinereovalba Fulgenzi & T.W. Henkel and F. ruschii A.C. Magnago.

Fistulinella jamaicensis is separated from F. gloeocarpa by its tiny basidiomes (pileus up to 1.8 cm diam., stipe 3 cm long, 3.5 mm wide), somewhat areolate pileus surface, smaller basidiospores [(9.5) 10–11 (12) × (4) 4.8–5.2 (μm, Qm = 2.0)], shorter hymenial cystidia (20–30 × 10–12(μm) with apical ampullaceous neck and apparently an absence of veil [20,22,108,109]. Lewis and Cibula [110] and more recently Bessette et al. [95] provided a re-description of F. jamaicensis from southern USA emphasizing characters such as a pileus up to 4.5 cm diam., pinkish or brownish pink than grayish to grayish brown piles with amber-yellow spots in age, stipe often with brownish scales and spores 8.5–14.5 × 4.5–6.5 μm. Given the several morphological discrepancies when a comparison is made with the original description by Murrill [108], we suspect it does not represent the same taxon.

Fistulinella venezuelae differs by the whitish to yellowish piles at the margin with yellow ochraceous to tawny center, ochraceous-ferruginous tints in the upper part of the stipe at maturity and pale yellowish to brownish gray mealy punctuations in the lower portion, white context with a pale ochraceous peripheral zone, absence of velar covering, weakly bitterish taste, elongate fusiform-cylindrical, much longer basidiospores [(12) 14.5–21.5 × 4.5–6 (6.5) μm, Qm = 3.2], generally longer hymenial cystidia (up to 93 μm long) usually exhibiting a long and slender neck, a cutis pileipellis with markedly narrower filamentous hyphae [(2) 3.5–10.5 μm wide] and growth in mountain environment in doubtful association with Alnus acuminata in Venezuela or in lowland vegetation in the Lesser Antilles (Martinique, Dominica) [14,20,51,55,111–113]. Additional collections of F. venezuelae have been made in Puerto Rico, Virgin Islands, and French Guyana (MycodPortal).

Guzmán described F. mexicana from evergreen lowland cloud forests in the Yucatan peninsula, southern-eastern Mexico [52] in putative association with Coccoloba spp. [114]. This species is distinguished from F. gloeocarpa by the yellowish brown or grayish, irregularly areolate pileus surface, presence of a colorless mucilaginous volva at the stipe base, slightly shorter basidiospores (8.1–12.2 × 4.1–5.9 μm), smaller, clavate pleurocystidia (24–43 × 5–8 μm), cheilocystidia none, cylindrical-globose caulocystidia (35–48 × 12–16(μm), narrower pileipellis hyphae (2.5–6.5 μm wide) and sometimes with an apparently lignicolous growth [20,30,52,114,115]. This species has most recently been check listed for the Mexican state of Quintana Roo by de la Fuente et al. [114].

Even if outwardly very similar, F. campinaranae and its var. scrobiculata Singer can be discriminated from F. gloeocarpa by the presence of a membranous but fugacious whitish ring on the stipe, slightly narrower, dextrinoid basidiospores [(11.5) 12–15
(18) × (3) 4–5 (6) µm, Qm = 3.3], narrower pilei-
pellis hyphae (3.4–10 µm broad), narrower hyphae of
lateral stratum (2–6 µm wide) in the hymenopho-
ral trama and the occurrence on rotting wood and
decayed stumps or less frequently on humus-sandy
soil in Brazilian Amazonian caatinga and campinar-
ana vegetation, in lowland Colombian rainforests
dominated by *Pseudomonotes* (Dipterocarpaceae)
and along the Brazilian coastal Atlantic Forest (Bahia) under leguminous trees (Fabaceae)
[20,31,42,57,116]. This species is also separated from
*F. gloeocarpa* based on molecular inference [42].
Regrettably, efforts for extracting DNA from either
the holotype collection (not located at INPA) and
paratype samples resulted unsuccessful [42].

*Fistulinella gloeocarpa* and *F. cinereolaeba* are two
look-alike species and phylogenetically most closely
related to each other, being sister species in the
molecular analysis (Figure 1). The latter species,
however, can be unraveled based on the stipe base
devoid of yellowish ochraceous spots, very finely
squamulose stipe surface, hymenophore and stipe
turning brownish when injured, decidedly longer
and slightly narrower, variably dextrinoid basidio-
spores [12.4–19.8 (24.8) × 3.7–4.9 (6) µm, Qm = 3],
acculate to cylindrical, narrower pleurocystidia
(37–61 × 3.7–6.2 µm), narrower pileiPELLIS hyphae
(2.4–7 µm broad), narrower hyphae of lateral stra-
tum (2–6 µm wide) in the hymenophoral trama and
the occurrence in Guyana in association with
*Dicymbe corymbosa* (Fabaceae subfamily
Caesalpinioideae) and along the coastal Atlantic
Forest in Brazil (Bahia) [27,116–118]. Magnago
[118] reports much broader pleurocystidia
(43–76 × 10–17 µm) for the Brazilian collections.

In some regards, *F. gloeocarpa* is also similar to
*F. ruschii*, however, corroborative features for distin-
guishing the latter species include the tomentose
and mostly dry, chestnut brown to orange-brown
pilule-brown pileus surface, cream pinkish stipe, NH₄OH staining
reddish orange and yellow on pileus and stipe,
respectively, longer and narrower basidiospores
[14–18 (22) × 4–5 µm, Qm = 3.4], presence of
broadly cylindrical, multiseptate pleurocystidia, nar-
rrower pileiPELLIS hyphae (4–11 µm broad), narrower
hyphae of lateral stratum (4–7 µm wide) in the
hymenophoral trama and the occurrence along the
Brazilian coastal Atlantic Forest under caesalpinoid
legumes (Fabaceae) and the Virgin Islands of the
Caribbean ([118] as “F. rhytidocystidiata Magnago &
M.A. Neves ad int.”, [42]; this study). Based on
morphological resemblance and preliminary phylo-
genetic inference (Figure 1) it appears quite possible
that *F. ruschii* and *F. venezuelae* might represent
the same taxon (collection TJB-8329 was formerly
identified by T.J. Baroni as *F. venezuelae*). Should this
conspecificity be confirmed, *F. venezuelae* would
have priority over *F. ruschii* having been described
previously than the latter species but further studies
are needed to elucidate their taxonomic relationships.

4. Discussion

The bulk of *Austroboletus* based on the data mining
and phylogenetic inference (Figure 1) indicates there
is a core for the genus, including the type species
(clade I). A very small number of species (including
*A. subflavidus*) attributed to *Austroboletus* that fall
outside the core (clades I and II), suggesting poly-
phyly, need a closer look and further analyses.
Accordingly, the disposition of *A. subflavidus*
ultimately results uncertain, nonetheless we feel that any
transfer to either a new genus or a new subgenus
(corresponding to clade II) would at present
be premature.

Macro- and micro-morphological features of
Dominican samples of *A. subflavidus* studied herein
consistently match those retrievable in the afore-
mentioned available literature but they display, as
already pointed out by Ortiz-Santana et al. [29]
based on Dominican and Belizean material, gener-
ally smaller dimensions and perhaps minor anatom-
ical differences with regard to the North American
populations, probably due to geographic distance
and different hosts or dissimilar climatic conditions.

Concerning biogeography, *A. subflavidus* shows a
broad distribution, spanning from warm temperate
to subtropical Atlantic regions of eastern, south-
eastern USA and Mexico south to the neotropical
countries of continental and insular Central
America [12,20,25,29,96] and northern South
America [31]. All reports of *A. subflavidus* from
outside its natural distribution range in the western
hemisphere (see, among others, [119–121]) should
be carefully re-assessed.

As far as the genus *Fistulinella* is concerned, since it
was first described from Martinique [55] *F.
 gloeocarpa* has not anymore been recollected else-
where, albeit Vasco-Palacios et al. [31] suggested a
possible occurrence in lowland *Pseudomonotes*
(Dipterocarpaceae) forests in Colombian Amazonia
but did not provide any convincing evidence.
Accordingly, the present account is the only docu-
mented record of *Fistulinella gloeocarpa* from out-
side the Lesser Antilles and the first from the
Dominican Republic. Indeed, genetic material gener-
it in this study match with a sequence deposited in
GenBank (GQ981503, collection KM162946) and
obtained by D.J. Lodge from the Bahamas islands,
 Further widening the distribution range of *F. gloeo-
carpa*. Moreover, three additional molecularly
unconfirmed records of this species have been spotted under *Coccoloba diversifolia* in Florida by J. Bolin (JAB211) and by Alan R. Franck (4662) in 2018 and published online in the mycological website Mushrooms Observer (https://mushroomobserver.org/observer/show_observation/359000).

Unfortunately, it has not been possible to either reexamine the holotype material or paratype collections made by Pegler in Martinique in the ‘70s preserved at the Royal Botanic Gardens Kew (K). On the other hand, no major morphological discrepancies can be observed when comparing the Dominican collections with the original description [55], despite its evident morphological variability. As a matter of fact, depending on the weather conditions and developmental stages, basidiomes of *F. gloeocarpa* may be extremely mutable concerning their morphological appearance. The most variable characters are the texture and color of the pileal surface; the surface is initially dark colored and finely rugulose-granulose but tends to become much paler, smooth, glutinous, and often typically rugulose-scrobulate. Neither in the original diagnosis nor in the comments of *F. gloeocarpa* mention is made about the presence of yellowish ochraceous spots at the stipe base [55]. However, a yellowish patch is clearly visible in one of the two color photographs (Pl. 19E-F) accompanying the original description, indicating that this chromatric trait was simply overlooked by Pegler. Likewise Pegler [55] did not report the presence of a ring on the stipe of *F. gloeocarpa* but it might have been removed accidentally by handling or simply gone unnoticed. However, the presence of a thick, persistent glutinous anulus obviously reflects an angiocarpic ontogenetic development and may determine, in addition to the mucilaginous volva reported for *F. mexicana* Guzmán (see below), an emendation of the diagnostic traits of *Fistulinella*, which was thought to be devoid of veils in all its representatives [16,32,55]. It would be advisable, however, to propose such an emendation only when the taxonomic limits of *Fistulinella* are better clarified.

In spite of the fact that morphological differences seem to justify specific separation of *F. gloeocapa* from the several closely allied species occurring in the same geographic macro-region, some of them (*F. jamaicensis*, *F. mexicana*, and *F. venezuelae*) are still lacking molecular confirmation and further research will be required to confirm their autonomous taxonomic status and mutual phylogenetic relationships. Especially *F. jamaicensis* and *F. mexicana* might finally turn out to be conspecific with *F. gloeocarpa* given morphological affinities and geographic proximity, but until sequenced material from Jamaica and Mexico is not available for comparison, it will be advisable to maintain these taxa as separate entities.

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