

Cantharellaceae of Guyana I: new species, combinations and distribution records of *Craterellus* and a synopsis of known taxa

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Abstract: Members of the Cantharellaceae (Cantharellales, Basidiomycota) are common ectomycorrhizal associates of the leguminous genus *Dicymbe* in the Pakaraima Mountains of Guyana. Eight distinct species or morphospecies currently are recognized in *Craterellus* Pers. or *Cantharellus* Adans. ex Fr. from Guyanese *Dicymbe*-dominated forests. We evaluated the systematics of these taxa with phylogenetic analyses of DNA sequence data from the nuclear ribosomal regions of the internal transcribed spacer (ITS) and 28S large subunit (LSU). The results of these analyses along with careful assessment of morphology let us describe two new species, *Craterellus atratoides* sp. nov. and *Craterellus strigosus* sp. nov., redescribe *Craterellus atratus* (Corner) Yomyart et al. based on new material from Guyana, and propose a new combination in *Craterellus* for *Cantharellus pleurotoides* T.W. Henkel, Aime & S.L. Mill. Macroscopic illustrations are provided for two additional cantharelloid morphospecies confirmed in *Craterellus*, as well as the regionally endemic *Cantharellus guyanensis* Mont. Macromorphological, micro-morphological and habitat data are provided for *C. atratoides*, *C. strigosus* and *C. atratus*, and ITS and LSU sequence data are provided for each of the eight known Guyanese taxa.

Key words: Cantharellaceae, chanterelles, *Dicymbe*, ectomycorrhiza, Guiana Shield, tropical fungi

INTRODUCTION

Species of the ectomycorrhizal (ECM) genera *Craterellus* Pers. and *Cantharellus* Adans. ex Fr. (Cantharellaceae, Cantharellales, Basidiomycota) are well represented in northern temperate zones but also occur in the tropics. Of the ~365 currently described species in the two genera worldwide (discounting infraspecific taxa), ~90 appear to have tropical distributions and most of these are paleotropical. In the more speciose *Cantharellus* ~300 species are described worldwide with ~76 tropical species; ~34 of these are known from tropical Africa, including the Congo Basin, miombo woodlands and Madagascar (e.g. Heinemann 1958, 1966; Eyssartier and Buyck 1999; Buyck et al. 2000) and ~31 from southeastern Asia, including Malaysia, Borneo, Philippines, Sri Lanka, Java, New Guinea and New Caledonia (e.g. Corner 1966, 1969; Ducouso et al. 2004). *Craterellus* has ~65 species described worldwide, with ~14 tropical species mostly described more than 45 y ago, half of which are paleotropical (Corner 1966).

Relative to the Paleotropics knowledge of Cantharellaceae in the Neotropics is limited. Seven species of *Cantharellus* and four of *Craterellus* were recorded from the South American lowland tropics, primarily from Brazil, Guyana, French Guiana and Venezuela (e.g. Montagne 1855, Corner 1966, Singer et al. 1983). Singer (1963) described an additional *Craterellus* species from montane Colombian oak woods. Several new species of *Cantharellus* recently have been described from Costa Rican or Colombian oak woods (Petersen and Mueller 1992, Eyssartier et al. 2003), Guyana (Henkel et al. 2006) and eastern Brazil (Petersen and Mueller 1992, Wartchow et al. 2012). In extratropical southern South America at least one species of *Cantharellus* is known from *Nothofagus* forests (Petersen and Mueller 1992). Only two new species of *Craterellus* have been described from the Neotropics in recent decades (Wu and Mueller 1995, Henkel et al. 2009).

In the central Guiana Shield of northeastern South America, *Craterellus* and to a lesser extent *Cantharellus* species are well represented in primary rainforests dominated by ECM canopy trees of the genus *Dicymbe* (Fabaceae subfam. Caesalpinioideae) (Henkel et al. 2012). Collecting during 15 y in Guyana's Pakaraima Mountains has documented *Cantharellus guyanensis* Mont., *Cantharellus pleurotoides* T.W. Henkel, Aime & S.L. Mill., and *Craterellus excelsus*

T.W. Henkel & Aime, plus five additional cantharelloid morphotaxa (Henkel et al. 2006, 2009, 2011). Four of these additional taxa are previously undescribed *Craterellus* species. Another taxon is similar to *Cantharellus atratus* Corner originally described from Brazil.

Herein we discuss the taxonomic placement of Guyanese Cantharellaceae based on morphology, molecular phylogenetic analysis and geographic distribution. We describe two new species, *Craterellus atratoides* sp. nov. and *Craterellus strigosus* sp. nov., and propose a new combination in *Craterellus* for *Cantharellus pleurotoides*. We also provide a redescription and new distribution record for *Craterellus atratus* (Corner) Yomyart et al. DNA sequence data from the internal transcribed spacer (ITS) and large subunit (28S) of the nuclear ribosomal repeat justify placement of each of these four species in *Craterellus*.

Two additional cantharelloid morphospecies occurred within *Craterellus*, and *Cantharellus guyanensis*, a new record for Guyana, was identified as the sole member of *Cantharellus* in the Guyanese assemblage. These three taxa are presented here in the phylogenetic analyses and illustrated. In a forthcoming paper we will formally describe or redescribe these taxa and provide a key to the Neotropical Cantharellaceae.

MATERIALS AND METHODS

Collections.—They were made during the May–Jul rainy seasons of 2000, 2001, 2002, 2006 and 2010 from the Upper Potaro River Basin, within a 4 km radius of a permanent base camp at 5°18'04.8"N, 59°54'40.4"W, 710 m (Henkel et al. 2011). Basidiomata were collected from forests dominated by *Dicymbe corymbosa* Spruce ex Benth. Macromorphological features of basidiomata were described fresh in the field. Colors were described subjectively and coded according to Kornerup and Wanscher (1978), with color plates noted in parentheses. Fungi were field-dried with silica gel.

Micromorphological features of fresh specimens were examined with an EPOI field microscope with light optics; dried specimens were examined with an Olympus BX51 microscope with light and phase contrast optics. For basidiospores, basidia, hyphal features and other structures and at least 20 individual structures were measured. Rehydrated fungal tissue was mounted in H₂O, 3% KOH and Melzer's solution. Line drawings were made with tracing paper with digital photomicrographs and modified with Photoshop CS5 (Adobe, San Jose, California). Guyana specimens were deposited in the following herbaria: BRG, University of Guyana; HSU, Humboldt State University; LSUM, Louisiana State University (Mycology); NY, New York Botanical Garden (Holmgren et al. 1990). Isotype specimens of *Cantharellus hystrix* Corner and *Cantharellus atratus* Corner were examined at the Royal Botanic Garden Edinburgh Herbarium (E). A specimen of *Craterellus orinocensis* Pat. & Gaillard was examined from the Tennessee Fungus Herbarium (TENN).

DNA extraction, amplification, sequencing and phylogenetic analyses.—DNA extractions of Cantharellaceae specimens from Guyana and elsewhere were performed with a QIAGEN DNeasy Plant Mini Kit (QIAGEN USA, Valencia, California; <http://www.qiagen.com/>). Polymerase chain reactions (PCR) and cycle sequencing were performed to obtain sequences of nuclear ribosomal DNA from the internal transcribed spacer (ITS) and the 28S large subunit (LSU), with primer pairs ITS1F/ITS4 (Gardes and Bruns 1993, White et al. 1990) and LR0R/LR5 (Vilgalys and Hester 1990) respectively. For PCR reactions the following reagents were combined per 25 µL reaction: 12.5 µL GoTaq Master Mix (Promega Corp., Madison Wisconsin), 9 µL PCR H₂O, 1.25 µL each of forward and reverse primers and 1 µL DNA template. Thermal-cycler protocols for the ITS were 94 C initial denature for 2 min. These steps repeated 32×: denature at 94 C for 30 s, anneal at 55 C 30 s, extension at 72 C 1 min; followed by a final extension of 72 C 5 min and a hold at 14 C indefinitely. The LSU PCRs followed the same protocols except with a 50 C annealing temperature.

Strong, single-copy PCR product was sequenced directly. Nearly 60% of the sequences generated had to be cloned due to intragenomic heterogeneity or weak amplification. Cloned PCR amplicons were generated with the TA or TOPO TA Cloning Kits (Invitrogen, Carlsbad, California). Fresh, cleaned PCR product was ligated to pCR 2.1 vectors that then were used to transform MAX efficiency DH5α-T1 chemically competent cells of *Escherichia coli*. Approximately 75 mL transformed cells in liquid SOC medium (Invitrogen) were incubated up to 24 h at 37 C on Luria-Bertani (LB) agar prepared with 50 µg/mL of kanamycin and 50 µL 50 mg/mL X-gal in dimethylformamide. Transformed colonies were screened with PCR with primers M13F and M13R (Invitrogen) followed by gel electrophoresis (1% agarose) with a 1 kb step ladder. A minimum of three amplicons of the expected size were chosen for sequencing.

Sequences were processed and assembled with Codon-Code Aligner 3.5.7 (CodonCode Corp., Dedham, Massachusetts; <http://www.codoncode.com/>). Assembled nucleotide sequence contigs of ITS and LSU regions were used in database BLAST queries of GenBank (www.ncbi.nlm.nih.gov/) and UNITE (unite.ut.ee/; Kõljalg et al. 2005). Query sequences that produced the closest match to sequences identified as either *Cantharellus* or *Craterellus* were used in preliminary alignments and phylogenetic analyses. Additional ITS and LSU sequences of primarily northern temperate Cantharellaceae species available on GenBank were included to assemble datasets with high intrafamilial inclusivity. Specimen and GenBank information for all taxa used in the study are provided (SUPPLEMENTARY TABLE I).

Initial alignment of datasets was performed with MUSCLE (Edgar 2004) followed by manual alignments using MacClade 4.07 (Maddison and Maddison 2005). Maximum likelihood (ML) and ML bootstrapping analyses were performed with RAxML (Stamatakis 2006), which was implemented on the CIPRES web portal (Miller et al. 2009) with 1000 bootstrap replicates to generate bootstrap statistics.

Bayesian analyses were performed with MrBayes 3.1.3 (Ronquist and Huelsenbeck 2003) implemented on the



FIG. 2. Previously described and undescribed Cantharellaceae taxa from Guyana. a. *Craterellus excelsus* T.W. Henkel & Aime (type; from Henkel et al. 2009). b. *Cantharellus guyanensis* Mont. (Henkel 9201). c. *Craterellus* sp. 1 (Henkel 9075). d. *Craterellus* sp. 2 (Henkel 9205). e. *Craterellus pleurotoides* (T.W. Henkel, Aime & S.L. Mill.) A.W. Wilson (Henkel 9220). Bars = 10 mm.

specimens identified previously as *Cantharellus guyanensis* (MCA981, MCA3112, TH9201) resolved as a strongly supported species-level taxon (100% MLB, 1.0 PP) within *Cantharellus* in both analyses (FIG. 1).

The *Craterellus* specimens in this study are represented by 28 ITS and 37 LSU sequences (SUPPLEMENTARY TABLE I). The genus was resolved in both ITS (64% MLB, 0.98 PP) and LSU (100% MLB and 1.0 PP) analyses and identified seven species-level taxa from the Guyanese specimens (FIG. 1). These included the previously described *C. excelsus*, which was resolved in close relationship to the undescribed morphospecies *Craterellus* sp. 1, each with three specimens. These two morphologically similar taxa are closely related as indicated by the supported monophyly of their clade under analysis of both ITS (70% MLB, 0.61 PP) and LSU (99% MLB, 1.0 PP). *Craterellus excelsus* and *Craterellus* sp. 1 each were supported with 100% MLB and 1.0 PP in both the ITS and LSU analyses (FIG. 1).

Another two-taxon clade was identified in *Craterellus* containing the morphologically similar, diminutive gray-brown species *C. atratus* and *C. atratoides*

sp. nov. (FIG. 1a, b). This clade was weakly supported in the ITS analysis (0.7 PP; FIG. 1a) but strongly supported in the LSU analysis (98% MLB, 1.0 PP; FIG. 1b). The three specimens of Guyanese *C. atratus* were morphologically consistent with the Brazilian *Cantharellus atratus* originally described by Corner (1966) and subsequently by Singer et al. (1983) and recently combined into *Craterellus* by Yomyart et al. (2011). The second species of this two-taxon clade, composed of three specimens, differs in several key morphological features from *C. atratus*, is distinct at the species-level in both phylogenies and is described here as a new species, *Craterellus atratoides* sp. nov.

Another species, represented by MCA1750 and TH9204, was not closely allied with any other species in both the ITS and LSU analyses (FIG. 1) and is described here as *Craterellus strigosus* sp. nov. At the species level, *Craterellus* cf. *atratus*, *C. atratoides* and *C. strigosus* each were supported at 100% MLB and 1.0 PP in both phylogenies.

Of the remaining two species resolved by the phylogenetic analyses, *Cantharellus pleurotoides* is



FIG. 3. Basidiomata of *Craterellus atratoides* (HOLOTYPE; Henkel 9232). Bar = 10 mm.

represented by specimens MCA3124 and TH9220 and supported at the species level with 100% MLB and 1.0 PP in both ITS and LSU analyses (FIG. 1). *Cantharellus pleurotooides* clearly was nested in *Craterellus*, forming the basis for its new combination reported below. The remaining taxon recognized by collectors as the morphologically distinct *Craterellus* sp. 2, represented by specimens MCA3186 and TH9250, was strongly supported at the species level in close relation to *C. pleurotooides* in the phylogenetic analyses (FIG. 1).

Preliminary work has determined that *Craterellus* sp. 1, allied with *C. excelsus*, and *Craterellus* sp. 2, allied with *C. pleurotooides*, are likely new to science; their formal description and a new description for *Cantharellus guyanensis* will be provided in a forthcoming paper. Basidiomata of all previously described and currently undescribed Guyanese Cantharellaceae are illustrated (FIG. 2).

TAXONOMY

Craterellus atratoides T.W. Henkel, Aime et A.W. Wilson, sp. nov. FIGS. 3, 4
MycoBank MB564237

Craterellus atratoides similis *Cantharelli atrati* Corner simili griseofusco colore, gracili cylindraceo stipite, bene definito pileo, et manifesto decurrentique hymenophoro; sed multo longiore stipite (25–70 vs. 8–30 mm), longioribus basidiis (79–111 vs. 54–67 μ m), in omnibus glabris et perforatis pileis stipitibusque, griseocaesio hymenophoro, et terresticola ubi fructificans differt.

Pileus 5–22.5 mm broad, 2.5–10 mm tall, initially planate with slightly downturned margin, with age becoming irregularly convex to uplifted with broadly

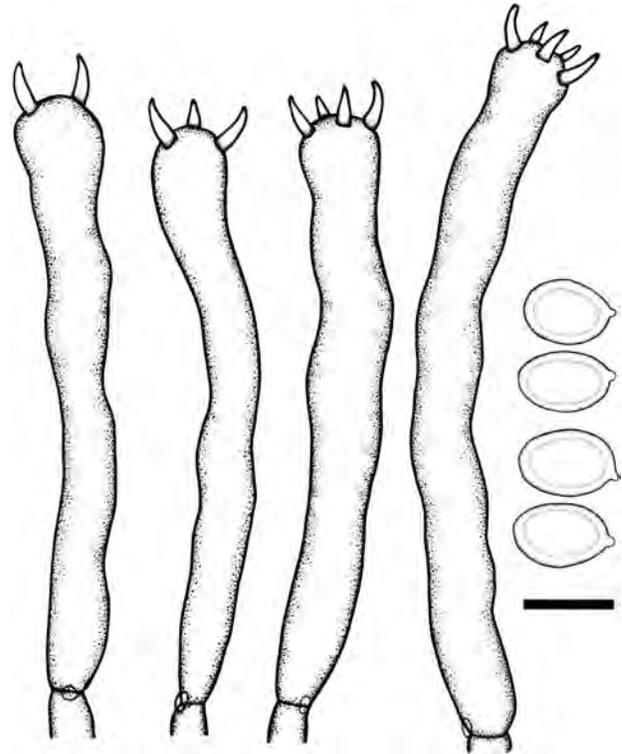


FIG. 4. Basidia and basidiospores of *Craterellus atratoides* (HOLOTYPE; Henkel 9232). Bar = 10 μ m.

wavy, down-curving margin, then strongly centrally depressed and perforate, dark grayish brown (8F4–9F4), transitioning to brown toward margin to light grayish tan at extreme margin; margin finely irregularly crenulate under hand lens, occasionally splitting, surface entirely glabrous, innately radially fibrillose under hand lens, moist; trama concolorous. Hymenophore continuous over lower side of pileus and descending stipe 1–5 mm with well demarcated but somewhat irregular lower edge, light bluish gray (19D2–19D3), initially smooth, thickening with age and becoming irregularly rugulose, hispid under hand lens due to projecting basidia, drying dark gray. Stipe 25–70 \times 1.5–3 mm, equal, flaring to 2–4 mm wide under fertile apex, concolorous (8F4–8F5) over apical two-thirds, somewhat lighter-concolorous over basal one-third, glabrous, with age flattening and subcanaliculate, basal mycelium wanting; trama concolorous with hollow core. Primordia filiform-acuminate, 0.5–1 mm tall. Odor none; flavor mildly fungoid, indistinct. Basidiospores (10)10.8–12(13) \times 7.1–9 μ m, Q range = 1.2–1.5, Q mean = 1.4, ellipsoid, smooth, hyaline to pale yellow in KOH, inamyloid, uniguttulate or rarely multiguttulate with granulose epiplasm; wall 0.3–0.5 μ m thick; hilar appendix 0.7–1 μ m long. Basidia (66)79–111 \times (4.9)7.4–9.9(10.1) μ m, 7.7–10.1(12.4) μ m wide at apex, 4.9–7.4 μ m at base,

subcylindrical, wall thin, hyaline to faintly pale yellow in KOH, devoid of obvious contents; sterigmata (2)3–4–5, (3.7)4.5–6.7(8.6) μm long, 1.5–2.5 μm wide at base, somewhat curving. Basidioles numerous, cylindrical, densely multiguttulate, pale yellowish brown in KOH. Cystidia none. Hymenium in transverse section 103–247 μm thick, light brown in KOH. Pileipellis a tightly interwoven mass of largely periclinal to subanticalinal hyphae, in mass brown in KOH; terminal elements of nearly equal lengths, undifferentiated and rounded at apex; individual hyphae hyaline to faintly yellow in KOH, devoid of obvious internal contents or occasionally guttulate; cells 24.7–64.2 \times 6.2–11.1 μm . Pileus tramal hyphae smooth, hyaline in KOH, branching occasionally, devoid of obvious internal contents or with numerous small guttules of various sizes; cells 44.5–108.7 \times 3.7–12.1(14.8) μm , somewhat inflating, constricted at septum when inflated; wall distinct, 1–1.2 μm thick; secondary septation absent. Stipitipellis composed of densely interwoven to subparallel hyphae arranged subanticalinally and with terminal elements often more periclinal, light brown in KOH; individual hyphae pale yellow brown in KOH, devoid of obvious internal contents; cells 37.0–74.1 \times 4.9–7.4 μm ; terminal elements undifferentiated and rounded at apex, or rarely slightly inflated near apex and subclavate. Stipe tramal hyphae smooth, hyaline to faintly yellow in KOH, occasionally branching, occasionally constricted at septum if inflated, devoid of obvious contents or scattered guttulate; cells 44.5–111.2 \times 3.7–11.1 μm . Clamp connections abundant on hyphae of all tissues.

Holotype: Henkel 9232 (BRG; ISOTYPE: HSU; NY)

Habit, habitat and distribution: Solitary to scattered on the root/humus mat of forest floor under *Dicymbe corymbosa* on white sand soils; known only from the type locality in the Upper Potaro Basin of Guyana.

Etymology: “atratooides” refers to the macroscopic resemblance of the species to *Craterellus atratus*.

Specimens examined: GUYANA: Region 8, Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt Ayanganna; 1.5 km northeast of base camp in mixed *Dicymbe-Micrandra* forest on white sand soils, 28 May 2010, Henkel 9232 (HOLOTYPE: BRG; ISOTYPE: HSU; NY); vicinity of base camp in *Dicymbe* forest, 17 Jun 2002, Henkel 8473 (BRG; HSU); ~3 km southwest of base camp vicinity of *Dicymbe* plot 3, 21 Jun 2000, Aime 1313 (BRG; LSUM). VENEZUELA. AMAZONAS: Atabapo, 9 Aug 1987, Halling 5462, *Craterellus orinocensis* Pat. & Gaillard!, (TENN 58453).

Commentary: *Craterellus atratooides* is recognized in the field by the slender, pileate-stipitate basidiomata with a long stipe relative to pileus width, dark grayish brown pileus and stipe with glabrous pelli, smooth to rugulose, decurrent, bluish gray hymenophore and

solitary to scattered terrestrial fruiting habit in *Dicymbe* forest occurring on white sand soils.

While the pileate-stipitate habit and presence of clamp connections might have led to placement of this species in *Cantharellus* under traditional morphological taxonomy (Corner 1966), our molecular data clearly indicate that *C. atratooides* is best placed in *Craterellus* (FIG. 1). The early development of conical primordia with subsequently perforate pilei in mature basidiomata of *C. atratooides*, in which the perforation is continuous with the hollow core of the stipe, are consistent with both the traditional concept of *Craterellus* (Corner 1966) and also the modern, in which absence of clamp connections was not considered universal in the genus (Dahlmann et al. 2000).

Species placed in *Cantharellus* subgen. *Phaeo-cantharellus* by Corner are gray to brown, have occasionally hollow stipes and clamp connections (Corner 1966). Among these, *C. atratooides* is most similar to the sympatric *C. atratus* reported here, sharing similar coloration, the slender cylindrical stipe, well defined pileus and sharply demarcated decurrent hymenophore. *Craterellus atratooides* is easily distinguished from *C. atratus* by its much longer stipe (25–70 vs. 8–30 mm), longer basidia (79–111 vs. 54–67 μm), consistently glabrous pileus and stipe, bluish gray hymenophore and terrestrial fruiting habit. The Bornean *Cantharellus fuliginosus* Corner is notably similar to *C. atratooides* in coloration and relative sizes of the elongated, subcylindrical stipe and well defined, small pileus with down-curved margin but differs fundamentally in its absence of clamp connections, smaller basidiospores (7.5–8.5 \times 5–6 vs. 10.8–12 \times 7.1–9 μm) and presence of well developed gill folds. *Cantharellus albomarginatus* (Coker) Corner from North Carolina, while dark overall, differs from *C. atratooides* in its blackish, subsquamulose pileus with abruptly white margin and smaller basidiospores (7–8.5 \times 4.2–6 vs. 10.8–12 \times 7.1–9 μm).

Among the few cantharelloid species described from the lowland South American tropics, *Craterellus orinocensis* resembles *C. atratooides* in basidioma size and coloration but differs in its more fully infundibuliform basidioma with fascicles of hairs on the stipe, much shorter (55–60 vs. 79–111 μm), consistently six-sterigmate basidia and lack of clamp connections (Patouillard and Gaillard 1888, Corner 1966, Singer et al. 1983). Examination of a collection identified as *C. orinocensis* from Atabapo, Venezuela (Halling 5462, TENN 058453) corroborated differences between *C. orinocensis* with *C. atratooides* in the former’s much broader infundibuliform stature and yellow-drying vs. gray-drying hymenium, lack of clamp connections and decidedly smaller basidiospores (6.2–8.6 \times 4.9–6.9 vs. 10.8–12 \times 7.1–9 μm).



FIG. 5. Basidiomata of *Craterellus strigosus* (HOLOTYPE; Henkel 9204). Bar = 10 mm.

Craterellus strigosus T.W. Henkel, Aime et A.W. Wilson, sp. nov. FIGS. 5, 6
MycoBank MB564238

Craterellus strigosus similis *Cantharelli hystricis* Corner parvo (<20 mm) pileo instructo cum densis strigosis trichomatibus, atrofuliginosis coloribus, laevi decurrenti hymenophoro, et aequali, parvo, solido stipite; sed griseofusco hymenophoro non violaceosuffuso, longiore stipite (13–33 mm vs. 12 mm), stipite cum dense-aggregatis strigosis pilis trichomatibus, et (2–3–4–5 vs. 4–6) sterigmatibus per basidium differt.

Pileus 4–18 mm broad, 2–9 mm tall, initially convex to subcampanulate and flattened across the apex, maturing to more regularly campanulate to planoconvex and then narrowly centrally depressed and somewhat out-flaring toward margin, dark brown (7F4–7F5), lighter concolorous at margin, lacking a distinct whitish band, surface with erect agglutinated strigose-fibrillose scales throughout, these more concentrated over the disk and 0.75–1 mm tall, otherwise appressed radially fibrillose to subrivulose near margin, submoist; extreme margin under hand lens finely crenulate and occasionally splitting; trama concolorous, subsolid. Hymenophore continuous over lower side of pileus and descending stipe 1–3 mm with well demarcated but irregular lower edge, gray with brownish overtones (7E2–7E3) throughout, smooth to finely rugulose, not thickening substantially, 0.1–0.3 mm thick in section, under hand lens hispid due to projecting basidia. Stipe 13–33 mm long up to hymenophore, 1–1.5 mm wide centrally, equal over basal four-fifths, flaring to 1.5–4 mm wide under fertile apex, concolorous with pileus, with dense, brown strigose scales throughout, scales 0.75–1.5 mm tall, lighter gray-brown at apices, slightly more concentrated near stipe base; trama concolorous, subsolid, lacking a hollow core. Primordia filiform-acuminate.

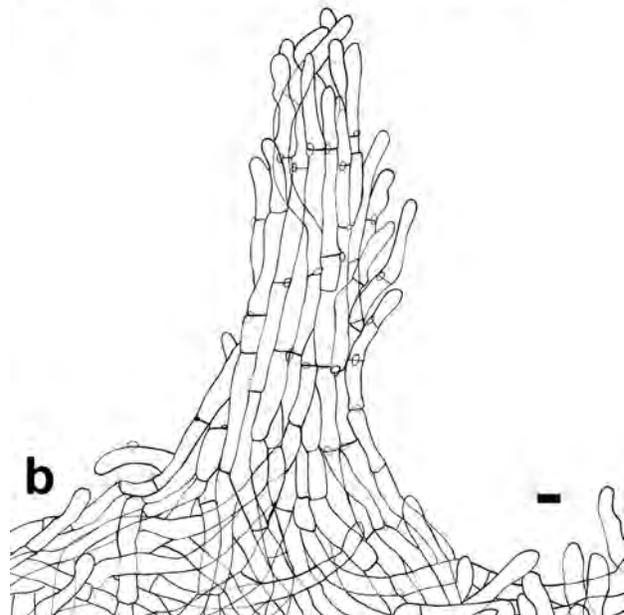
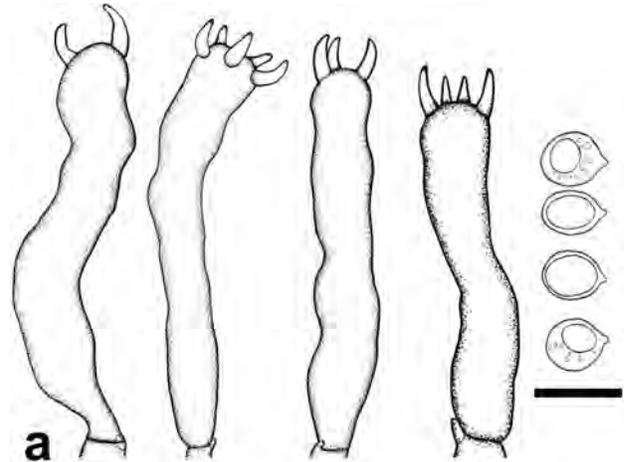


FIG. 6. Micromorphological features of *Craterellus strigosus* (HOLOTYPE; Henkel 9204). a. Basidia and basidiospores. b. Fasciculate hyphae of the stipitipellis. Bars = 10 μ m.

Odor none; flavor minimal, indistinct. Drying non-hygrophanous to dark brown. Basidiospores (7)8–10 \times 5.9–8(8.5) μ m, Q range = 1.1–1.5, Q mean = 1.3, broadly ellipsoidal, smooth, hyaline in KOH, inamyloid, with one large guttule with uniform, finely granulose contents, infrequently multiguttulate; wall 0.4–0.8 μ m thick; hilar appendix 0.9–1.2 μ m long. Basidia 52–70 \times 5.2–9.9 μ m, (6.2)7.2–10.4(12.1) μ m wide at apex, (3)3.7–9.6 μ m at base, subcylindrical to nearly cylindrical, hyaline in KOH, devoid of obvious contents or occasionally finely granulose; sterigmata 2–3–4–5, (3.7)4.4–5.7(7.4) μ m long, 1.5–2.5 μ m wide at base, somewhat curving. Basidioles numerous, cylindrical, non-guttulate, with opaque, finely granulose contents.

Cystidia none. Hymenium in transverse section 74–284(469) μm thick. Pileipellis of largely repent to subantical hyphae interspersed with erect fascicles, hyphae pale tan-yellow in KOH, slighter darker in mass, cylindrical, cells (14.8)29.6–54.3(79) \times 4.9–12.3 μm ; fascicles anticlinal, 135–220 \times 50–116 μm , composed of 10–15 parallel to slightly intertwining hyphae, these of same dimensions as repent pileipellis elements, terminal cells undifferentiated. Pileus tramal hyphae smooth, hyaline in KOH, branching occasionally, interwoven, lacking obvious internal contents, cells 29.6–100 \times 4.9–19.5 μm , moderately inflating, narrowing or constricting near septa when inflated; secondary septation absent. Stipitipellis hyphae mainly repent between abundant erect hyphal fascicles; fascicles (128)198–260 \times (29)46–54(66) μm , composed of 12–20 parallel hyphae, frequently narrowing over apex to 3–4 hyphae wide, apex overall subacuminate or blunt with terminal cells of equal length, cells 12.3–39.5 \times 3.7–6.2 μm , pale yellow-tan in KOH, devoid of obvious internal contents. Stipe tramal hyphae in mass pale brown in KOH, densely interwoven, branching frequently, individual hyphae pale yellowish in KOH, uninflated. Clamp connections abundant on hyphae of all tissues.

Holotype: Henkel 9204 (BRG; ISOTYPE: HSU; NY)

Habit, habitat and distribution: Scattered or in small troops on humus deposits or directly on live bark on trunks of *Dicymbe corymbosa* in Guyana. Known only from the type locality in the Upper Potaro Basin, Guyana.

Etymology: “Strigosus” (L. adj. A) = covered with bristle-like hairs, referring to the prominent vestiture on the pileus and stipe.

Specimens examined: GUYANA: Region 8, Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt Ayanganna; ~1 km west of base camp on trail to *Dicymbe* plot 3, 20 May 2010, Henkel 9204 (HOLOTYPE: BRG; ISOTYPE: HSU; NY); 3 km southwest of base camp in *Dicymbe* plot 3, 2 Jun 2001, Aime 1750 (BRG; LSUM). SINGAPORE: Botanic Garden, 11 Nov 1934, *Cantharellus hystrix* Corner!, leg. E.J.H. Corner, s.n., typus herb. Cantab. (ISOTYPE: E, in formalin-alcohol).

Commentary: Molecular data clearly indicate that *Craterellus strigosus* is best placed in *Craterellus*, although the species exhibits several traditional diagnostic features of *Cantharellus*, including the presence of clamp connections, a solid stipe and stipitate/pileate stature. The presence of filiform-acuminate primordia in *C. strigosus*, however, is consistent with both traditional and modern concepts of *Craterellus* (Corner 1966, Dahlman et al. 2000). In Guyana, *C. strigosus* has been confirmed via root molecular analysis as an ECM symbiont of *D. corymbosa*, *Dicymbe altsonii* Sandw. and *Aldina insignis* (Benth.) Endl. (Smith et al. 2011).

Craterellus strigosus is similar to *Cantharellus hystrix* Corner, known from the type collection from Singapore (Corner 1966 p 66–67) and Singer’s collection, identified as *C. hystrix*, from central Brazil (Singer et al. 1983 p 14–15). All share the small (<20 mm) pileus beset with dense, erect strigose scales, somber colors, the smooth hymenophore that is decurrent, and the equal, small, solid, concolorous stipe. *Craterellus strigosus* differs from *C. hystrix* in its grayish brown hymenophore lacking in violaceous tones, longer stipe (13–33 mm vs. 12 mm), the presence of densely aggregated strigose scales on the stipe and the number of sterigmata per basidium (2–3–4–5 per basidium vs. 4–6). Examination of the isotype of *C. hystrix* at E confirmed the more diminutive basidioma size, paucity of strigose scales on the stipe and predominance of sterigmata numbers ≥ 4 . Wartchow (unpubl) recently reported a new collection identified as *C. hystrix*, consisting of a single basidioma, from the Mata Atlantica in Pernambuco, Brazil, which is in some agreement with the Guyanese *C. strigosus*, although the sterigmata number again varies, 2–3–4–5 in the Guyana material vs. (4–5)6 in the Pernambuco collection. While the morphological differences between *C. strigosus* and *C. hystrix* may seem slight, we feel justified in erecting the new species because: (i) no convincing material of *C. hystrix* has emerged from Asia since the single type collection of Corner’s from Singapore in 1934; (ii) sequence data is unobtainable from Corner’s pickled type; (iii) there are morphological differences; and (iv) the likelihood of conspecificity between the Asian and Neotropical material is low. Determining whether the Guyanese *C. strigosus* is conspecific with Brazilian material identified as *C. hystrix* by Singer and Wartchow will require new collections from the previous localities and generation of appropriate sequence data for assessment.

Among Guyanese cantharelloids *C. strigosus* superficially resembles *C. atratus*, also reported here, in its small basidioma, colorations and decurrent, sharply demarcated hymenophore. *C. strigosus* can be distinguished macroscopically from *C. atratus* by its darker brown and regular occurrence of dense, erect scales covering the pileus, especially over the disk, and over the surface of the stipe. In addition *C. strigosus* dries in a non-hygrophanous manner to dark brown, unlike the hygrophanous *C. atratus*. *Craterellus atratus* also lacks the finely granulose, opaque contents of the basidiospores and basidioles regularly apparent in *C. strigosus*. In addition, molecular phylogenetic analyses clearly separate the two species (FIG. 1).

The sympatric *C. atratoides* is also a small, somber, fuliginous *Craterellus*, but differs from *C. strigosus* in lacking strigose scales on its glabrous pelli, having a



FIG. 7. Basidiomata of *Craterellus atratus* (Henkel 9203). Bar = 10 mm.

much longer stipe (25–70 vs. 13–33 mm), longer basidiospores (10.8–12 vs. 8–10 μm), longer basidia (79–111 vs. 52–70 μm) and a consistently terrestrial fruiting habit.

Craterellus atratus (Corner) Yomyart, Watling, Phosri, Piapukiew & Sihanonth Figs. 7, 8
 = *Cantharellus atratus* Corner. A monograph of cantharelloid fungi. Ann Bot Mem 2:62. nom. nov.

Pileus 9–32 mm broad, 5–7 mm tall, initially campanulate and flattened across apex, broadly campanulate with out-flaring margin to nearly plane with age, centrally depressed throughout development, initially dark brown (6F5–7F5) throughout with slightly lighter narrow marginal zone, with age light brown or paler (6E3–6E4, 5D4 or 4C2) over central two-thirds, transitioning toward margin to a nearly off-white marginal zone 1–2 mm wide; surface initially with scattered erect, low strigose scales, these less prominent with age becoming nearly glabrous, otherwise appressed radially fibrillose, canescent, and finely rugulose under hand lens, moist; margin finely and irregularly crenulate to nearly crowded-setose, occasionally splitting with age; trama concolorous, 1–1.5 mm thick, thickening somewhat above stipe, subsolid. Hymenophore continuous over lower side of pileus and descending stipe 2–9 mm with well demarcated but irregular lower edge, initially light gray or ashy gray (6B1–7B1, 4B1), slightly lighter with advanced age, initially nearly smooth to finely rugulose under hand lens, with age thickening substantially and rugose to radially canaliculate, finely hispid under hand lens due to projecting basidia; 0.3–0.5 mm thick in longitudinal section. Stipe 8–30 mm long up to hymenophore, 0.75–2 mm broad centrally, subequal, subsolid-flexible, 2.5–6 mm broad under

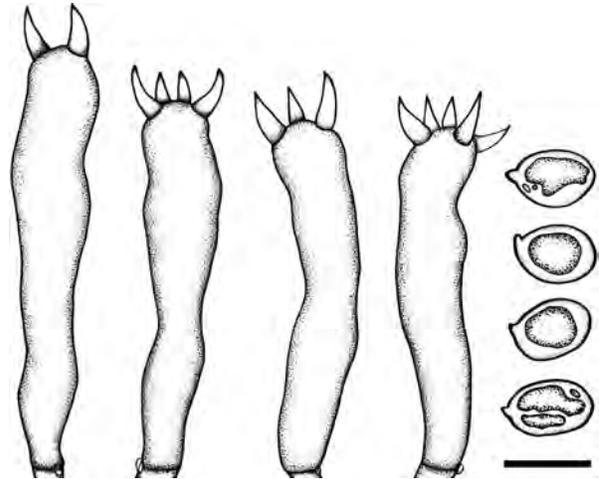


FIG. 8. Basidia and basidiospores of *Craterellus atratus* (Henkel 9203). Bar = 10 μm .

descending hymenophore near apex, concolorous with pileus throughout development, generally glabrous macroscopically, under hand lens with minute, scattered, erect-acuminate strigose scales, less pronounced with age, more concentrated and persistent toward base; trama solid, concolorous, lacking a hollow core. Primordia filiform-acuminate. Odor none; flavor mild initially, slightly acrid with time. Drying hygrophanous to light gray, hymenophore becoming dull tan. Basidiospores 9–10(11) \times 7–8.5(9) μm , Q range = 1.1–1.4, Q mean = 1.2, subellipsoid, smooth, hyaline in KOH, inamyloid, with one large guttule, rarely multiguttulate; wall 0.5–1.0 μm thick; hilar appendix 0.8–1.0 μm long. Basidia 54–67(73) \times 7.4–9.9(11.9) μm , 9.9–12.4(13.6) μm wide at apex, 4.9–7.7(9.4) μm at base, subcylindrical, wall thin, hyaline in KOH, devoid of obvious contents or uniformly granulose and opaque, rarely with numerous small guttules; sterigmata (2)3–4–5, (3.7)4.9–7.4(10.1) μm long, 1.2–2.5 μm wide at base, somewhat curving. Basidioles numerous, cylindrical, with numerous opaque guttules of various sizes. Cystidia none. Hymenium in transverse section 113–358 μm thick. Pileipellis of largely periclinal elements, in mass light grayish brown in KOH, arranged sporadically into subantical to antical hyphal fascicles; fascicles 259–424 \times 96–163 μm , of 25–40 loosely to more closely packed hyphae, these subparallel to slightly interwoven, light grayish tan in KOH, subacuminate or rounded over collective apex; individual hyphae of a fascicle uninflated, cells 22–56 \times 4.9–8.6 μm , terminal cells undifferentiated and rounded at tips, hyaline to pale grayish yellow in KOH, thin-walled. Pileus tramal hyphae smooth, hyaline in KOH, branching occasionally, lacking in

obvious internal contents, cells 37.1–113.6(190) × 4.7–14.4 µm, inflating somewhat, occasionally narrowing to rarely constricted near septa; secondary septation absent. Stipitipellis essentially a cutis interspersed with frequent erect hyphal fascicles; fascicles 102–320 × 40–80 µm, of 6–15 loosely to more closely packed hyphae, subacuminate or rounded over collective apex; individual fascicle hyphae uninflated, cells 17–30 × 7–10 µm, terminal cells undifferentiated, hyaline to pale yellow in KOH, thin-walled. Stipe tramal hyphae interwoven with longitudinal orientation, branching occasionally, hyaline in KOH, thin-walled, devoid of obvious contents, cells 27.2–59.3(69.2) µm × (5)7.4–9.9 µm, barely inflating; secondary septation absent. Clamp connections abundant on hyphae of all tissues.

Habit, habitat and distribution: Scattered or in small troops on humus deposits or directly on live bark on trunks of *D. corymbosa* in Guyana. Also known from eastern and central Brazil and possibly southeastern Asia.

Specimens examined: GUYANA: Region 8, Potaro-Siparuni: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt Ayanganna; 0.75 km west of Potaro base camp on line to *Dicymbe* plot 3, 20 May 2010, *Henkel 9203* (BRG; HSU); 100 m west of old Ayanganna airstrip 20 May 2000, *Aime 990* (BRG; LSUM); ~3 km southeast of base camp in *Dicymbe* plot 1, 28 May 2001, *Aime 1070* (BRG; LSUM). BRAZIL: Rio de Janeiro, Corcovado, 20 Nov 1948, *Cantharellus atratus* Corner!, leg. E.J.H. Corner, typus in herb. Cantab. (ISOTYPE: E, in formalin-alcohol).

Commentary: *Craterellus atratus* is easily recognized in Guyana's *Dicymbe* forests by its small gray, centrally stipitate-pileate basidiomata lacking pronounced strigose scales and occurring in small to large troops in elevated positions on humic deposits on the trunks of *D. corymbosa*. Molecular data clearly indicate that the Guyanese taxon is best placed in *Craterellus*, although it exhibits several traditional diagnostic features of *Cantharellus*, including the presence of clamp connections, a solid stipe and stipitate/pileate stature. The presence of filiform-acuminate primordia in *C. atratus*, however, is consistent with both traditional and modern concepts of *Craterellus* (Corner 1966, Dahlman et al. 2000). We are applying the name *Craterellus atratus* to the Guyana taxon due to its regional co-occurrence and high morphological congruence with previously described Brazilian collections of *Cantharellus atratus*.

The Guyana collections of *Craterellus atratus* agree well with the Brazilian type description for *Cantharellus atratus* of Corner (1966 p 62), confirmed by our examination of the isotype at E. The salient macro-morphological features are consistent, including the small, campanulate pileus becoming nearly plane with out-flaring margin, pileus surface with varying

densities of short, erect scales, the smooth, thickening hymenophore that is decurrent and abruptly demarcated from the stipe, the slender, short stipe, a general concurrence of all colors, and gregarious fruiting habit. Corner's original description indicated a lack of stipe strigose scales, which are variably present in the Guyana material, depending on basidioma age and in greater concentration toward the stipe base. Another description of the species by Singer et al. (1983 p 13–15), based on the type and a second Brazilian collection, notes that stipe strigose scales are present on some basidiomata, and all other morphological features are in concordance with the Guyana material. While it is impossible to determine the ECM host plants associated with the type collection from southeastern Brazil, Singer's second collection came from campinarana forests on sand soils with leguminous ECM host trees similar to those occurring in Guyana (Singer et al. 1983).

Craterellus atratus from Guyana microscopically agrees with Brazilian material placed in *C. atratus* by Corner and Singer for most features. The Guyana material deviates from Corner's Brazilian type description in having consistently shorter basidia (54–67[73] vs. 70–100 µm), which was confirmed by microscopic examination of the isotype. Corner noted that the Brunei collection also cited in the original description has basidia 47–65 µm long, in concordance with our material and also with the short basidia (41–48 µm) recorded by Singer for his Brazilian material. Comparison of *C. atratus* with the superficially similar, sympatric *C. strigosus* can be found above.

Craterellus atratus is one of the most frequently fruiting ECM fungi in Guyana's monodominant *D. corymbosa* forests, with basidiomata occurring in 66.2% of 630 sampling quadrats over 7 y of rainy season surveys (Henkel et al. 2012). The discovery of *Craterellus atratus* in Guyana provides a putative range extension of nearly 1000 km to the north from the central Brazilian Amazon (Singer et al. 1983). In Guyana, *Craterellus atratus* has been confirmed via root molecular analysis as an ECM symbiont of *D. corymbosa*, *D. altsonii* and *A. insignis* (Smith et al. 2011).

Craterellus pleurotoides (T.W. Henkel, Aime & S.L. Mill.) A.W. Wilson, comb. nov.

≡ *Cantharellus pleurotoides* T.W. Henkel, Aime & S.L. Mill. 2006. Mycol Res 110, p 1410.

Commentary: The combination is warranted due to the molecular phylogenetic placement of *C. pleurotoides* within the *Craterellus* lineage of the Cantharellaceae (FIG. 1). *Cantharellus pleurotoides*, with a highly reduced, pleurotoid basidioma atypical of the

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