New species of *Clavulina* (Cantharellales, Basidiomycota) with resupinate and effused basidiomata from the Guiana Shield

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Abstract: Three new species of *Clavulina* (Cantharellales, Basidiomycota) are described from rainforests dominated by ectomycorrhizal trees of the leguminous genus *Dicymbe* (Fabaceae subfam. Caesalpinioideae) from the central Guiana Shield. Species of *Clavulina* typically form branched, coralloid basidiomata with amphigenous hymenia. However, the three species described here form resupinate or effusocoralloid basidiomata, macromorphological forms previously unknown in *Clavulina*. Macromorphological, micromorphological, habitat and DNA sequence data are provided for each new species. Micromorphological features and DNA sequence data from the second largest subunit of DNA-dependant RNA polymerase II (*rpb2*) and internal transcribed spacer (ITS) and large subunit (28S) of the ribosomal repeat justify placement of these new species in *Clavulina*. Comparisons with described *Clavulina* species and other resupinate taxa within the Cantharellales are provided.

   Key words: Agaricomycetes, cantharelloid clade, ectomycorrhizal fungi, fungal systematics, Guyana, *Membranomyces*, Neotropics

INTRODUCTION

The genus *Clavulina* Schroet. (Clavulinaceae, Cantharellales, Basidiomycota) has been traditionally characterized by branched coralloid basidiomata with amphigenous, thickening hymenia, basidia with two incurved sterigmata and often with postpartal septa,

and smooth, hyaline, guttulate basidiospores (Corner 1950, 1970; Petersen 1988a). While primarily tropical, species of *Clavulina* also occur in temperate zones (e.g. Corner 1950, 1970; Petersen 1988a; Henkel et al. 2005, 2011a). Recent molecular studies have confirmed that *Clavulina* is a monophyletic lineage within the Cantharellales (Thacker and Henkel 2004, Moncalvo et al. 2006, Olariaga et al. 2009) and that species in this genus form ectomycorrhizas with diverse host plants (e.g. Tedersoo et al. 2003, 2007, 2008, 2010a; Moyersoen 2006; Peay et al. 2010; Kennedy et al. 2011; Smith et al. 2011).

In the tropical forests of the Guiana Shield *Clavulina* species comprise much of the macrofungal diversity associated with ectomycorrhizal (ECM) leguminous canopy trees of the genera *Dicymbe* (subfam. Caesalpinioideae) and *Aldina* (subfam. Papilionoideae; Henkel et al. 2002, 2005, 2011a; Thacker and Henkel 2004; Smith et al. 2011). Of the nearly 170 morphospecies of putatively ECM fungi collected as sporocarps over 13 y in this region approximately 25 are *Clavulina* spp. (Henkel et al. 2011b). Although only approximately 75 species of *Clavulina* have been described globally, ongoing descriptions of new species from Guyana are rapidly expanding the number of known species (Corner 1950, 1970; Thind 1961; Petersen 1983, 1985, 1988a, b; Thind and Sharda 1984; Roberts 1999; Thacker and Henkel 2004; Henkel et al. 2005, 2011a; Duhem and Buyck 2007; Douanla-Meli 2007; Trappe and Castellano 2007).

The majority of known *Clavulina* species conform to the traditional generic diagnosis. However several species from the tropics deviate macro- or microscopically from the classic *Clavulina* morphology. A number of species with unbranched basidiomata have been described from the paleo- and Neotropics as well as Australasia (Corner 1950, 1970; Petersen 1983, 1985, 1988a; Henkel et al. 2005). One highly unusual species with infundibuliform, *Craterellus*-like basidiomata, *Clavulina craterelloides* Thacker and T.W. Henkel, was described from Guyana (Thacker and Henkel 2004). Petersen (1988b) noted that the putatively diagnostic postpartal basidial septa may occur sporadically or be absent on individual basidiomata and he downplayed the generic importance of this character. The basidium with two incurved, horn-shaped (''cornute'') sterigmata has long been considered the key feature allowing segregation of *Clavulina* from coralloid genera such as *Clavaria*

(Agaricales; Corner 1950, Petersen 1988a). However even bisterigmate basidia are not universal in *Clavulina*, as exemplified by the 2–3(4)-sterigmate *Clavulina amazonensis* Corner and 4–5(6)-sterigmate *Clavulina nigricans* Thacker and T.W. Henkel. These species were assigned to *Clavulina* due to their cornute sterigmata and congruent basidiospore morphology, and their generic placement has been supported by DNA sequence analyses (Corner 1970, Petersen 1988b, Thacker and Henkel 2004, Henkel et al. 2011a).

Molecular studies have shown that the resupinate basidioma form has arisen independently in many of the major clades of Agaricomycetes, including the *Cantharellales* (Hibbett and Binder 2002; Binder et al. 2005; Larsson 2004, 2007). This paper reports the first discovery of *Clavulina* species that have highly reduced, resupinate or effused basidiomata. While the taxa described herein are reminiscent of species of *Membranomyces* Jülich, a resupinate genus with close affinities to *Clavulina* (Larsson et al. 2004), multiple lines of evidence are provided for their placement in *Clavulina*. *Clavulina cerebriformis* sp. nov., *Clavulina cinereoglebosa* sp. nov. and *Clavulina effusa* sp. nov. are described with basidiomata that are either resupinate or effuso-coralloid and with 2–4 sterigmata per basidium.

Macromorphological, micromorphological, habitat and DNA sequence data are provided for each of these extraordinary species. Phylogenetic analysis based on *rpb2* and 28S rDNA sequences was used to assess the generic placements and infrageneric affinities of these new taxa.

MATERIALS AND METHODS

Collections.—Collections were made during the May–Jul rainy seasons 2001, 2003 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 2003). Basidiomata were collected from forests dominated by *Dicymbe corymbosa* Spruce ex Benth. Macroscopic 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, sterigmata and hyphal features at least 20 individual structures were measured. Rehydrated fungal tissue was mounted in H_2O, 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). Specimens are deposited in these herbaria (Holmgren et al. 1990): BRG, University

of Guyana; HSU, Humboldt State University; NY, New York Botanical Garden; DUKE, Duke University Herbarium.

DNA extraction, amplification, sequencing and phylogenetic analyses.—DNA extractions were performed on basidioma tissues that were dried rapidly in the field with silica gel. Tissues were homogenized with sterile forceps or a micropestle, and DNA was extracted with the Extract-N-Amp DNA kit (Sigma-Aldrich Ltd., St Louis, Missouri) or with the DNeasy Plant Mini Kit (QIAGEN, Valencia, California). The ITS1-5.8s-ITS2 (ITS) and partial large subunit (28S) of the ribosomal DNA were amplified with forward primers ITS1F and LROR and reverse primers ITS4B, LR3, LR5F in various combinations with protocols of Vilgalys and Hester (1990), Gardes and Bruns (1993) and Tedersoo et al. (2008). The second largest subunit of DNA-dependant RNA polymerase II (*rpb2*) gene was successfully amplified from three *Clavulina* specimens with general primers RPB2-5F and RPB2-7R (Liu et al. 1999). Using these three sequences and the single *Clavulina rpb2* sequence from GenBank (DQ366286) we designed a set of two forward and three reverse *rpb2* primers with enhanced specificity for *Clavulina*: RPB2Clav100F (5′-GACCAAAAGAARTT-CATGTCTGC-3′), RPB2Clav96F (5′-GGGGYGACCAAAA-GAARTTCA-3′), RPB2Clav792R (5′-CRGGSGTCATACAGAT-CATCA-3′), RPB2Clav800R (5′-AGGTCATCRGGSGTCATACA-3′), and RPB2Clav938R (5′-GRATCTCRCAATGCGTCCA-3′). The following PCR conditions were employed for amplification of *rpb2* amplicons: 94 C for 5 min followed by 25 cycles of 30 s at 94 C, 30 s at 63 C and 2 min at 72 C. This was followed by 11 additional cycles of 30 s at 94 C, 30 s at 45 C and 2 min at 72 C with a final extension of 7 min at 72 C. PCR products were viewed on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, Oregon). Amplicons were cleaned with Exonuclease I and shrimp alkaline phosphatase (Glenn and Schable 2005) and sequenced with Big Dye 3.1 (Applied Biosystems, Foster City, California) with the same primers used for amplification. Sequencing reactions were cleaned and processed on an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, California) at the Duke University Genome Sequencing & Analysis Core Facility. Newly generated sequences were edited in Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, Michigan) and initially blasted in GenBank (http://www.ncbi.nlm.nih.gov/) with BLASTN to assess generic affinities. All DNA sequences generated in this study have been submitted to GenBank and are provided here (TABLE I) along with additional Cantharellales sequences accessed from GenBank and used in the phylogenetic analyses.

The ITS region is highly variable among *Clavulina* species and cannot be aligned reliably across the genus. Therefore this region was used only to assess similarity among the two closely related new species, *C. cinereoglebosa* and *C. effusa*. To examine differences among these species we performed neighbor joining analysis based on 749 bp of the ITS region from two specimens of each species. For phylogenetic analysis at a broader scale we used both the 28S rDNA and *rpb2*. The 5.8s region was not used because it is highly conserved and provides almost no phylogenetic signal within *Clavulina*. Both *rpb2* and 28S rDNA were available for all taxa used in the analysis except for

Table I. Taxa and accession numbers for sequences used in the phylogenetic analyses

Taxon	Voucher number	28S	RPB2	Source location
Tulasnella violea	DAOM 222001	AY293216	–	?
Tulasnella violea	GEL 2561	–	DQ898768	Germany?
Cantharellus appalachiensis	GRSM 77088	DQ898690	DQ898748	Tennessee, USA
Craterellus tubaeformis	TM 0268	DQ898741	DQ898749	Ontario, Canada
Hydnum albomagnum	PBM 2512	AY700199	DQ234553	Massachusetts, USA
Hydnum repandum	KHL 8552	AF347095	–	Sweden
Hydnum repandum	DSH 97-320	–	AY218489	Massachusetts, USA?
Membranomyces delectabilis	KHL11147	AY586688	–	Sweden
Clavulina cerebriformis	MCA4022	JN228222	JN228233	Guyana
Clavulina sp.	MB 03-034	AY745694	DQ366286	Massachusetts, USA
Clavulina cf. *cristata*	MES426	JN228225	JN228239	Yunnan, China
Clavulina cf. *cinerea*	MES427	JN228226	JN228240	Yunnan, China
Clavulina cinerea	DUKE9351	JN228216	JN228251	North Carolina, USA
Clavulina cristata	DUKE9312	JN228215	JN228250	North Carolina, USA
Clavulina cinerea	JKU9	JN228228	JN228242	California, USA
Clavulina cristata	JKU8	JN228227	JN228241	California, USA
Clavulina monodiminutiva	TH8738	DQ056372	JN228237	Guyana
Clavulina caespitosa	TH8709	DQ056370	JN228234	Guyana
Clavulina humicola	TH8737	DQ056367	JN228244	Guyana
Clavulina tepurumenga	MCA3116	HQ680363	JN228248	Guyana
Clavulina sprucei	TH9122	JN228223	JN228236	Guyana
Clavulina sprucei	MCA3989	HQ680352	JN228235	Guyana
Clavulina cf. *connata*	TH9586	JN247429	JN228247	Guyana
Clavulina nigricans	TH8284	AY391719	JN228238	Guyana
Clavulina ornatipes	TH9598	JN228229	JN228243	California, USA
Clavulina cinereoglebosa	TH8561	JN228232	JN228246	Guyana
Clavulina effusa	TH9193	JN228230	JN228245	Guyana
Clavulina amazonensis	TH8742	HQ680361	JN228249	Guyana

Membranomyces delectabilis, for which only 28S rDNA was available. DNA sequences were compiled in Mesquite 1.1 (Maddison and Maddison 2006) and aligned with MUSCLE (Edgar 2004). Initial analyses did not indicate evidence of strong discordance in phylogenies based on the individual genes, and therefore we concatenated sequences of the two genes for the final analysis. For the two-gene phylogenetic analyses we used an alignment consisting of 784 bp of *rpb2* and 827 bp of 28S for a total length of 1611 bp. After ambiguous regions were excluded 1351 bp remained for analysis. Of these 679 characters were constant whereas 440 bp were parsimony informative. Parsimony analysis was performed with the default settings and parsimony bootstrapping with 1000 replicates in PAUP 4.0a112 (Swofford 2002). Maximum likelihood analysis was performed with the GTR + I + G model with the software package Garli 0.951 (Zwickl 2006). Maximum likelihood bootstrapping was performed with 100 replicates using the default settings in Garli 0.951.

Results

Phylogenetic analyses.—For the two-gene phylogenetic analyses, parsimony analysis produced 19 equally parsimonious trees of 1790 steps. The maximum likelihood analysis produced a phylogeny with a likelihood score of ln −9330.89423 (Fig. 1). The 28S-*rpb2* analysis supports placement of *C. cerebriformis*, *C. cinereoglebosa* and *C. effusa*, as well as *Membranomyces delectabilis*, within an otherwise monophyletic *Clavulina* within the Cantharellales and also suggests that resupinate forms have at least two independent derivations (Fig. 1).

Neighbor joining analysis.—Neighbor joining analysis based on 749 bp of the ITS region from two specimens of each species indicated clear separation of *C. cinereoglebosa* and *C. effusa* (Supplementary Fig. 1).

Taxonomy

Clavulina cerebriformis Uehling, Aime et T.W. Henkel, sp. nov. Figs. 2, 3
MycoBank MB561188

Species nova basidiomatibus resupinatis et basidiis 2–3(4)-sterigmaticis Clavulinae cinereoglebosae Uehling, Aime et T.W. Henkel similis, sed basidiis brevioribus 32–46 (non 62–75 um), parietibus hyphalibus angustioribus, 0.3–0.7 (non 0.8–1.0 um), basidiosporis subglobosis appendiculo hilari distincte breviore praeditis differt.

Basidiomata sub-globose to sub-cerebriform erumpent structures, these sometimes fusing, unevenly
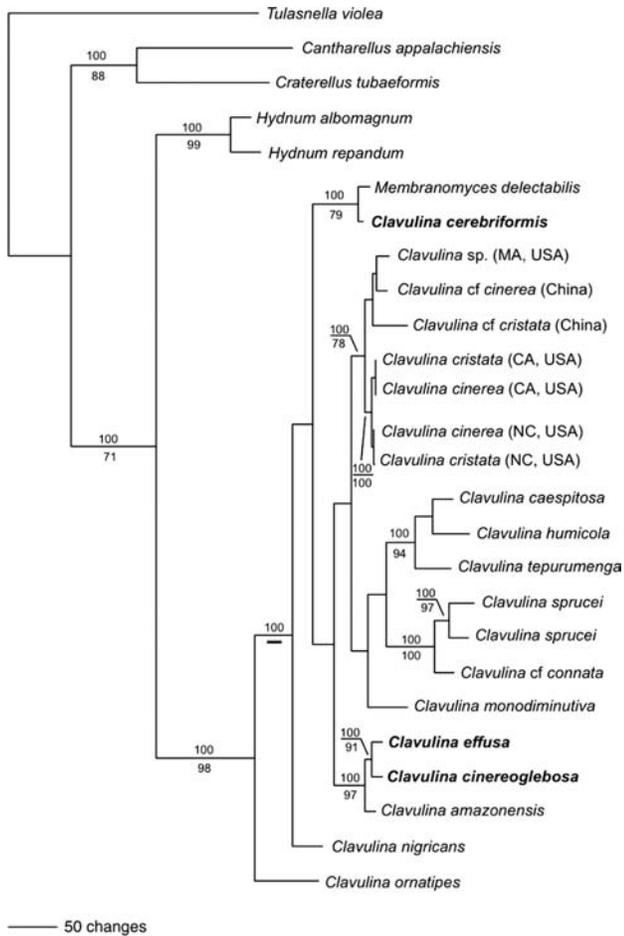
FIG. 1. Maximum likelihood phylogeny (ln −9330.89423) depicting phylogenetic relationships of *Clavulina* species and other Cantharellales taxa based on combined analysis of *rpb2* and 28S rDNA sequences. The three newly described species, *C. cerebriformis*, *C. cineroglebosa* and *C. effusa*, are indicated in boldface. Maximum parsimony bootstrap values are above the nodes and maximum likelihood bootstrap values are below the nodes. *Tulasnella violea* was used as outgroup taxon. For *Membranomyces delectabilis* only 28 S data were available for analysis.

FIG. 2. Basidiomata of *Clavulina cerebriformis* (HOLO-TYPE; *Aime 4022*). Bar = 10 mm.

grayish green globules; postpartal septa absent; sterigmata cornute, 4–5(6) µm long, 2–3(4) per basidium. Basidioles numerous. Cystidia absent. Hymenium covering entire exposed surface, in longitudinal section up to 0.5 mm thick. Tramal hyphae smooth, lacking internal contents, hyaline, uninflated; cells 12–25 × 3.0–6.0(7.0) µm; wall thin, 0.3–0.7 µm wide. Clamp connections absent.

Holotype. *Aime 4022* (BRG; ISOTYPE HSU)

Habit, habitat and distribution. Fruiting as a cluster on particulate humic deposits ∼ 1 m high on trunk of *D. corymbosa* in *Dicymbe*-dominated forest, in May during the early rainy season. Known only from the type locality in the Upper Potaro River Basin of Guyana.

Etymology. Cerebriformis (Latin adj. B) = brain-like; referring to the macromorphology of the basidiomata.

Specimen examined. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, 5°18′04.8″N, 59°54′40.4″W, 710–750 m; vicinity of base camp, on trunk of *D. corymbosa*, 24 May 2010, *Aime*

undulating and somewhat pitted, clustered and sessile with subtending resupinate portions directly on humic deposits in trunk cavities of *D. corymbosa*; individual basidiomata 0.5–3.0 mm wide, 0.5–1.0 mm thick, pale yellowish cream (2A1–2A2), moist, hispid under hand lens. Odor minimal; flavor not obtained. Basidiospores (6.5)7–8(8.5) × 6.5–7.5 µm (mean Q = 1.11), subglobose, smooth, hyaline in H_2O, pale blue gray in KOH, inamyloid, with one translucent guttule; wall 1 µm thick; hilar appendage 0.5–0.75 µm long. Basidia (28)32–46(51) × 7–9(10) µm, tapering gently downward over lower half to 3–7(7.5) µm wide at base, subcylindrical, constricted at basal septum, with
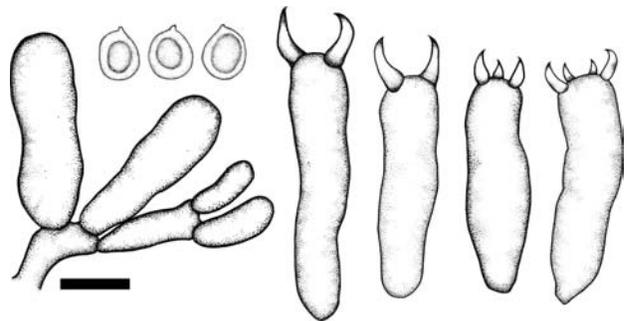

FIG. 3. Basidioles, basidia and basidiospores of *Clavulina cerebriformis* (HOLOTYPE; *Aime 4022*). Bar = 10 µm.
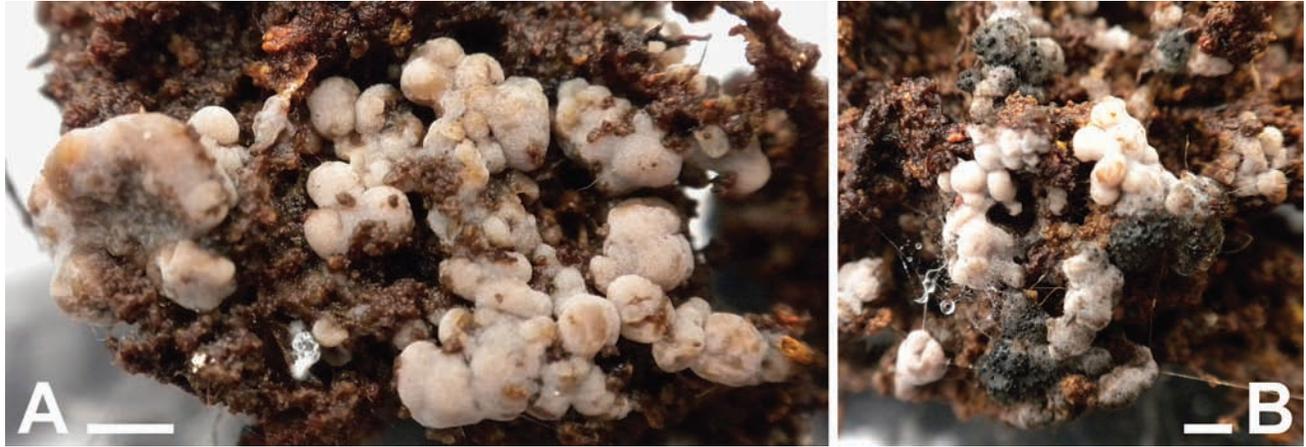
FIG. 4. Basidiomata of *Clavulina cinereoglebosa*. A. HOLOTYPE (*Aime 4023*). B. Basidiomata with sordarialean parasite. Bar = 10 mm.

4022 (HOLOTYPE BRG; ISOTYPE HSU; LSU GenBank JN228222; *rpb2* GenBank JN228233).

Commentary. Clavulina cerebriformis is distinguished by its small, whitish, sub-globose to sub-cerebriform, hispid basidiomata spread over particulate humic substrata on *Dicymbe* trunks. The non-coralloid habit separates *C. cerebriformis* from all previously described species of *Clavulina.* Micromorphology of basidia and basidiospores and the 28S-*rpb2* combined analysis support the generic placement of *C. cerebriformis* (FIGS. 1, 3). Additionally the ECM status of *C. cerebriformis* is supported by recovery of *C. cerebriformis* ITS rDNA sequences from ECM roots of *D. corymbosa* (Smith et al. 2011).

Clavulina cinereoglebosa, a second resupinate species described here, can be differentiated from *C. cerebriformis* by its grayish, less convoluted basidiomata, thicker hyphal walls (0.8–1.0 vs. 0.3–0.7 µm), and sub-lacrimiform basidiospores with a longer hilar appendage (1.0–1.3 vs. 0.5–0.75 µm). Among other resupinate members of the cantharelloid clade *C. cerebriformis* is similar to species of *Membranomyces* in having pale, repent basidiomata, unclamped hyphae, and hyaline, smooth basidiospores. *Clavulina cerebriformis* differs from *M. spurius* (Bourdot) Jülich in having a more convoluted, three-dimensional basidioma and 2–3(4)-sterigmate basidia that are shorter (32–46 µm) than the regularly four-sterigmate, 35–75 µm long basidia reported for *M. spurius* by Jülich (1975). *Membranomyces delectabilis* (H.S. Jacks.) Kotir. & Saaren. differs from *C. cerebriformis* in having an adnate, non-erumpent basidioma, much narrower basidia (4–5 vs. 7–10 µm) that are (1)2–4-sterigmate, with much longer sterigmata (up to 9 µm; Kotiranta and Saarenoksa 1993). The two described species of *Membranomyces* are known from north temperate and boreal forests (Eriksson and Ryvarden 1976, Kotiranta

and Saarenoksa 1993), whereas *C. cerebriformis* is known only from a *Dicymbe*-dominated forest in Guyana.

Among species of the resupinate genus *Clavulicium* Boidin that have bisterigmate basidia *Clavulicium macounii* (Burt) J. Erikss. & Boidin is similar to *C. cerebriformis* in its resupinate, yellowish white basidiomata but differs in having regularly clamped hyphae, occasional unisterigmate basidia with nearly straight sterigmata and longer basidiospores (9–12 vs. 7–8 µm; Eriksson and Ryvarden 1973).

Clavulina cinereoglebosa Uehling, Aime et T.W. Henkel, sp. nov. FIGS. 4, 5
MycoBank MB561189

Species nova Clavulinae effusae Uehling, Aime et T.W. Henkel colore basidiomatico et hispiditate superficiali partis effusae similis, sed parietibus hyphalibus crassioribus, 0.8–1.0 (non 0.3–0.5 um) et basidiosporis sublacrimiformibus loco depresso suprahilari distincto carentibus differt.

Basidiomata resupinate-erumpent, somewhat fusing and patch-like, exposed on humic particulate mass at base of *D. corymbosa*, also occurring on stem of living seedling near ground level; entire fructification ∼ 12 mm² in area, erumpent portions broadly rounded, 0.5–1 mm diam, flesh gray (6B1–6B2), moist, pruinose to downy under hand lens, subtended by thin, gray (6B1) resupinate areas that fuse and bind particulate substrata; some basidiomata parasitized by a dark sordarialean ascomycete. Odor and flavor not obtained. Basidiospores 6–7(7.5) × 5.5–7 µm (mean Q = 1.10), sub-lacrimiform, tapering evenly toward hilar appendage, smooth, hyaline in H₂O, hyaline to pale yellowish in KOH, inamyloid, with one translucent guttule; wall ∼ 1 µm thick; hilar appendage 1.0–1.3 µm long. Basidia (56)62–75(83) × 4–6(7) µm centrally, 6–8(8.5) µm across apex, 3–4 µm
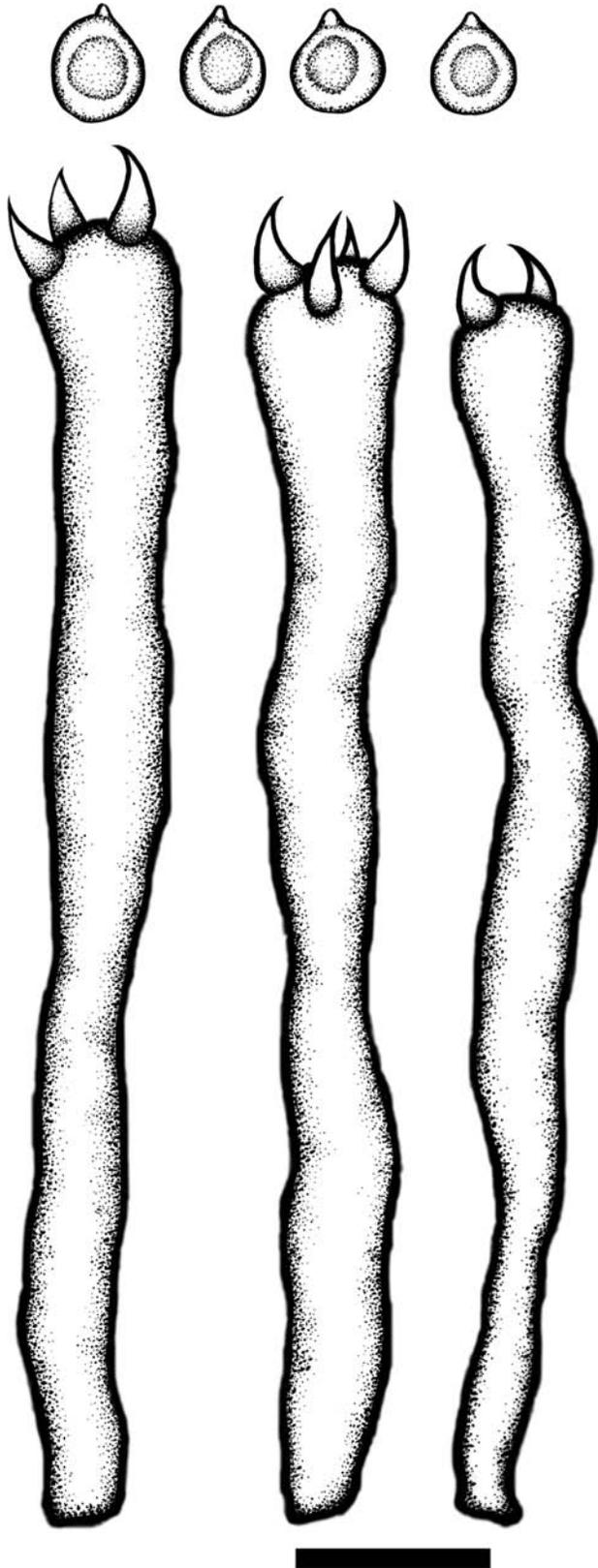
FIG. 5. Basidia and basidiospores of *Clavulina cinereoglebosa* (HOLOTYPE; *Aime 4023*). Bar = 10 μm.

at base, sub-cylindrical to sub-clavate, tapering evenly downward over the lower two-thirds, with pale grayish green contents, relatively thick-walled at 0.8–1.0 μm, postpartal septa absent; sterigmata cornute, 4–5 μm long, 2–3(4) per basidium. Basidioles numerous. Cystidia absent. Hymenium covering entire exposed surface, in longitudinal section 0.3–0.5 mm thick. Tramal hyphae smooth, wall 0.8–1.0 μm thick, lacking internal contents, uninflated; cells 15–45 × 3–6 μm. Clamp connections absent.

Holotype. *Aime 4023* (BRG; ISOTYPE HSU)

Habit, habitat and distribution. Occurring as a small, patchy fructification on humic materials at base of mature *D. corymbosa* or on seedling stem in *Dicymbe*-dominated forest, in May during the early rainy season. Known only from the type locality in the Upper Potaro River Basin of Guyana.

Etymology. Cinereo (Latin adj. A) = gray + glebosus (Latin adj. A) = lumpy; referring the color and form of the basidiomata.

Specimens examined. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, within 4 km radius of 5°18′04.8″N, 59°54′40.4″W, ~ 710 m; ~ 2 km SE base camp near *Dicymbe* plot 2, on seedling stem, 14 Jul 2003, *Henkel 8561* (BRG; HSU); ITS GenBank JN228217; LSU GenBank JN228232; *rpb2* GenBank JN228246; 1 km SE base camp on Benny's Ridge, on humus at base of mature *D. corymbosa*, 24 May 2010, *Aime 4023* (HOLOTYPE BRG; ISOTYPE HSU; LSUM).

Commentary. *Clavulina cinereoglebosa* is characterized in the field by grayish, sessile, semi-erumpent basidiomata clustered on organic substrata, with pruinose surfaces due to projecting basidia. As with *C. cerebriformis*, *C. cinereoglebosa* is unlike any previously described *Clavulina* species due to its non-coralloid fructification and can be easily differentiated from *C. cerebriformis* as outlined above. Micromorphology of basidia and basidiospores and the 28S-*rpb2* combined analysis support the generic placement of *C. cinereoglebosa* (FIGS. 1, 5).

Clavulina effusa, also described here, combines an effused, fertile basidioma giving rise to vertical, branched, coralloid projections. *Clavulina cinereoglebosa* is similar in color and surface hispidity to the effused portion of *C. effusa* but differs micromorphologically in having consistently thicker hyphal and basidial walls (0.8–1.0 vs. 0.3–0.5 μm) and sublacrimiform basidiospores that are slightly but consistently shorter (6–7 vs. 7–8.5 μm) as opposed to the sub-ellipsoid to pyriform basidiospores with a distinct suprahilar depression of *C. effusa*. While only two collections of *C. cinereoglebosa* are at hand, neither give indication of erect, coralloid development in the manner of *C. effusa*. The ITS sequences of *C. cinereoglebosa* and *C. effusa* clearly separated the two species at approximately 94% similarity across the

entire ITS region (ITS1-5.8s-ITS2; SUPPLEMENTARY FIG. 1).

Clavulina cinereoglebosa is superficially similar to *Membranomyces delectabilis* in its resupinate, grayish basidiomata. However *C. cinereoglebosa* differs in having shorter basidiospores (6–7 vs. 8–11 µm) and longer basidia (62–75 vs. 40–55 µm). It also lacks the unisterigmate basidia found in *M. delectabilis* (Kotiranta and Saarenoksa 1993).

Clavulina effusa Uehling, T.W. Henkel et Aime, sp. nov.   FIGS. 6, 7

MycoBank MB561190

Species nova magnitudine basidiomatis, colore et habitu fructificationis Clavulinae monodimunitivae T.W. Henkel, Meszaros et Aime similis, sed basidiomatibus effuso-coralloideis et septationibus regularibus secondariis differt.

Basidiomata effuso-coralloid, with fertile hymenium on both effused and upright branching portions; fruiting in troops with upright caespitose clusters on decaying sticks, wood fragments and litter on forest floor; clusters 5–15 mm wide, 6–20 mm tall; originating as gray with orangish pink undertones (6D3–6E3) resupinate mats on undersides of organic substrata, surface of mat hispid under hand lens and fertile before origin of erect portions; thickening into discrete lumpy protuberances that develop on upper side of mat anticlinal to gravity; these eventually projecting upward into primordia that are monopodial, filiform and sharply acuminate, grayish orange (5B3–5B4) initially, slightly lighter at extreme tips, 2–6 × 0.2–0.4 mm before branching, becoming hymenial early except for extreme tips and gradually concolorous with resupinate portion; primordia emerging and ultimately orienting upward, eventually branching once to twice, first dichotomously and then polychotomously, 11–20 mm tall × 2–7 mm wide across branches, branch tips acuminate throughout development; hymenium brownish orange to grayish brown (7C3–7D3), amphigenous, thickening with age to 0.1–0.2 mm, superficially fusing the branches, hispid under hand lens. Odor pleasantly fragrant-fungoid; flavor minimal. Basidiospores (6.5)7–8.5(9) × 6–7 µm (mean Q = 1.16), sub-ellipsoid to sub-pyriform with a distinct suprahilar depression, smooth, hyaline in H_2O, pale yellowish hyaline in KOH, inamyloid, with one or multiple translucent guttules; wall 1 µm thick; hilar appendage 0.5–1 µm long. Basidia (43)52–78(84) × 4–6(7) µm centrally, 5–8(8.5) µm across apex, (2)3–4.5(7) µm wide at base, sub-clavate, tapering downward evenly over lower two-thirds, with grayish green contents; wall 0.3–0.5 µm thick; postpartal septa absent; sterigmata cornute, 4–7 µm long, 2–3 per basidium. Basidioles numerous. Cystidia none. Hymenium in transverse section
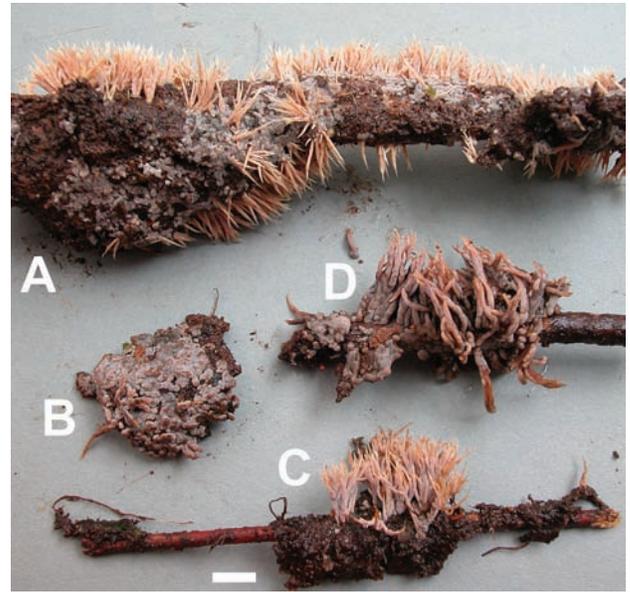

FIG. 6. Basidiomata of *Clavulina effusa* (HOLOTYPE; *Henkel 9193*). A. Troops of developing coralloid basidiomata and resupinate portion on humus-covered stick. B. Fertile resupinate portion with primordium initials. C. Developing coralloid basidiomata with young hymenia. D. Mature coralloid basidiomata with thickened hymenia. Bar = 10 mm.

100–150 µm thick. Tramal hyphae smooth, uninflated, wall 0.3–0.5 µm wide, lacking internal contents; cells 13–48 × 3–5(6) µm. Clamp connections absent.

Holotype. *Henkel 9193* (BRG; ISOTYPE HSU; DUKE; NY)

Habit, habitat and distribution. In effuso-coralloid troops of caespitose clusters on forest floor litter in *D. corymbosa*-dominated forests, fruiting in May–Jul rainy season. Known only from the type locality in the Upper Potaro River Basin of Guyana.

Etymology. Effusus (Latin part. A) = spreading, flat; referring to the distinctive resupinate portion that gives rise to erect coralloid fructifications.

Specimens examined. GUYANA. REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, within 4 km radius of 5°18′04.8″N, 59°54′40.4″W, ~ 710 m; ~ 3 km southwest of Potaro base camp in *Dicymbe* plot 3, 5 Jul 2001 *Henkel 8244* (BRG; HSU); vicinity of Potaro base camp, 26 July 2001, *Henkel 8386* (BRG; HSU); ~ 1.5 km SE base camp near *Dicymbe* plot 1, 16 May 2010, *Henkel 9193* (HOLOTYPE BRG; ISOTYPE HSU; DUKE; NY); ITS & LSU GenBank JN228230; *rpb2* GenBank JN228245.

Commentary. *Clavulina effusa* is characterized in the field as grayish orange to grayish brown and formation of fertile resupinate and upright coralloid portions in troops on various types of litter substrata of the forest floor. The effuso-coralloid macromorphology separates *C. effusa* from all previously described species of
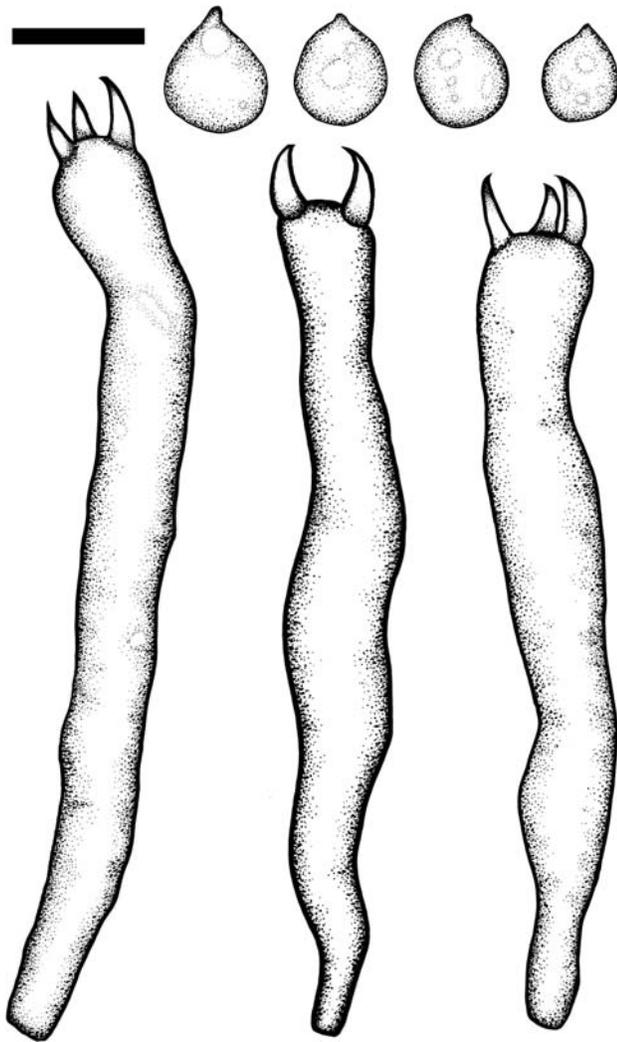
FIG. 7. Basidia and basidiospores of *Clavulina effusa* (HOLOTYPE; *Henkel 9193*). Bar = 10 μm.

Clavulina. Micromorphology of basidia and basidiospores and the 28S-*rpb2* combined analysis support the generic placement of *C. effusa* (FIGS. 1, 7).

Among previously described *Clavulina* species from Guyana *C. effusa* shares branched basidiomata and gray hymenia with *C. tepurumenga* T.W. Henkel & Aime. However *Clavulina tepurumenga* is easily distinguished morphologically by its much larger basidiomata (40–166 mm × 16–68 mm across the branches) that fruit directly from the mineral soil and lack a resupinate portion (Henkel et al. 2011a). *Clavulina monodimunitiva* T.W. Henkel, Meszaros & Aime has similarly small, gray basidiomata that fruit in troops on decaying sticks, wood and leaves. However the basidiomata of *C. monodimunitiva* do not branch, lack a resupinate portion and the basidia have postpartal septations (Henkel et al. 2005). The resupinate portion of *C. effusa* is macroscopically

similar to *Membranomyces delectabilis* but differs in having shorter basidiospores (7–8.5 vs. 8–11 μm) and shorter sterigmata (4–7 vs. 9 μm). *Clavulina effusa* also lacks the unisterigmate basidia reported for *M. delectabilis* by Kotiranta and Saarenoksa (1993).

DISCUSSION

The discovery of *Clavulina* species with resupinate or effused basidiomata expands the generic concept of *Clavulina* and likely will stimulate discovery of additional morphologically reduced taxa. The presence of these unusual morphological forms of *Clavulina* in the Guiana Shield is not surprising. This region is home to several other enigmatic and morphologically distinctive *Clavulina* species, such as *C. amazonensis, C. nigricans* and *C. craterelloides* (Petersen 1988b, Thacker and Henkel 2004, Henkel et al. 2011a). The 28S-*rpb2* analysis reported here supports placement of *C. cerebriformis, C. cinereoglebosa* and *C. effusa* within a monophyletic *Clavulina* and also suggests that resupinate forms have at least two independent derivations (FIG. 1). The analysis also indicates an apparent sister relationship between the resupinate *C. cinereoglebosa* and effuso-coralloid *C. effusa*. Additionally resupinate taxa currently classified in *Membranomyces* may be placed more appropriately in *Clavulina* (see *M. delectabilis*, FIG. 1), as corroborated by Larsson et al. (2004) and Tedersoo et al. (2010b). However additional type studies of *M. spurius* and *M. delectabilis* are necessary before such conclusions can be drawn.

Another interesting aspect of the topology (FIG. 1) includes the close relationships among north temperate *C. cristata* and *C. cinerea*, which apparently constitute species complexes across these regions (Olariaga et al. 2009). Also suggested here is that north temperate *Clavulina* taxa are not closely allied with those from the Guiana Shield. The lack of support at various nodes, however, along with the modest number of taxa included, limit the utility of the current phylogeny for making assertive inferences about character-state evolution or phylogeography. More data and taxa clearly are needed to elucidate the potentially complex infrageneric structure of *Clavulina*. This project is ongoing (Uehling, Smith and Henkel unpubl).

The high diversity of *Clavulina* species known from basidiomata collections in Guyana is corroborated by high *Clavulina* diversity from ECM root sequences. A recent molecular study of ECM fungi on *Dicymbe* and *Aldina* roots in Guyana found that *Clavulina* was one of the most species-rich groups, with greater than 20 ITS rDNA types detected at one site (Smith et al. 2011). More than half of these species are currently

known only from ECM root tip sequences. This phenomenon may extend widely across the South American tropics; in Venezuela *Clavulina* species were found to be prominent mycobionts with the dipterocarp host *Pakaraimaea* (Moyersoen 2006) and in Ecuador with species of *Coccoloba* (*Polygonaceae*) and *Neea* (Nyctaginaceae; Tedersoo et al. 2010a). Clearly ongoing mycological explorations in tropical South America will yield further new discoveries in *Clavulina*.

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