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A new *Cantharocybe* from Belize with notes on the type of *Cantharocybe gruberi*

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Abstract: A new species of *Cantharocybe*, *C. brunneovelutina*, is described and illustrated from the Orange Walk District of Belize. The species is characterized by the subvelutinous brown pileus, brownish gray stipe and microscopically by cheilocystidia that have prong-like appendages. Analysis of nLSU rDNA sequences relates this species to *Cantharocybe gruberi*, the sole member of a previously monotypic genus of uncertain placement. We also provide a microscopic description and illustrations of the type collection of *Cantharocybe gruberi* and compare the two species. Morphological comparisons among *Cantharocybe* and its sister genus, *Cuphophyllus* (= *Camarophyllus*), and allied genera are discussed.

Key words: Agaricomycetes, Hygrophoraceae, Neotropical fungi, rDNA systematics, taxonomy, Tricholomataceae

INTRODUCTION

Recent efforts to document the macrofungi in Belize have led to the discovery of a rich mycoflora of potentially undescribed species and even genera (e.g. Ortiz-Santana et al. 2007; Baroni et al. 2007, 2008, 2009; Ginns et al. 2010). One such unusual fungus was discovered near the La Milpa Field Station within the Rio Bravo Conservation Area on part of the uplifted limestone Rio Bravo escarpment. This part of northern Belize, located at the base of the Yucatan Peninsula, lies across the border from the Calakmul Biosphere Reserve in Mexico, and the two reserves are part of the same biogeographic region. The habitat is predominantly lowland dry limestone forest amid Mayan ruins, with rare *Pinus* and *Quercus* on sand ridges.

The species defied easy placement to genus. Although several macromorphological features were reminiscent of genus *Tricholomopsis* Singer, such as the robust, highly velutinous basidiomes, this placement was rejected based on micromorphology. Subsequent DNA sequencing and analysis of the nuclear large subunit (LSU) ribosomal RNA gene indicated close affinity with *Cantharocybe gruberi* (A.H. Sm.) H.E. Bigelow & A.H. Sm. In this paper we describe and illustrate the new taxon, *Cantharocybe brunneovelutina*, emend genus *Cantharocybe*, provide a redescription of *C. gruberi* and discuss the relationship between *Cantharocybe* and other genera near the base of the Hygrophoraceae, especially *Cuphophyllus* (Donk) Bon and *Ampulloclitocybe* Redhead, Lutzoni, Moncalvo and Vilgalys. We refer to *Cuphophyllus* instead of *Camarophyllus* in this paper because the former is a valid name for the genus typified by *Agaricus pratensis* Schaeff., whereas the latter is not a valid name for this genus and can be applied only to a different group at an infrageneric rank in genus *Hygrophorus* s.s. typified by *H. caprinus* (formerly *Agaricus camarophyllus* Alb. & Schwein.; Donk 1962, Courtecuisse and Fiard 2005, Melot 2005, Young 2005).

MATERIALS AND METHODS

Material was collected during a biotic survey and inventory of basidiomycete fungi in Belize and the Dominican Republic (NSF DEB-0103621 grant 2001–2005 to Timothy J. Baroni and the Research Foundation, SUNY at Cortland, and co-PI D.J. Lodge at USDA-FS). Colors are descriptive except for capitalized names in parentheses that are Ridgway (1912) colors as reproduced by Smithe (1975). Cross, radial and tangential sections were cut by hand and mounted in 3% KOH or Melzer's reagent. Size ranges and mean length, width and Q (Q = length divided by width) of spores were based on ocular micrometer measurements of 20 spores.

We explored placement of *C. brunneovelutina* with analyses of the nuclear large subunit (LSU) ribosomal RNA gene. DNA was extracted from dried tissue with the UltraClean Plant DNA Isolation Kit (MoBio Laboratories Inc., Solana Beach, California) and the first ~ 1200 bp of the LSU ribosomal DNA amplified and sequenced with LSU4-B (Aime and Phillips-Mora 2005) and LR6 (Moncalvo et al. 1995) with the parameters of Aime and Phillips-Mora (2005). Sequences were edited in Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, Michigan) and alignments were constructed in MAFFT 6.5 using Q-INS-I (Katoh & Toh 2008), then assessed visually in Se-Al 2.0a11 (Andrew Rambaut, Dept. Zoology, University of Oxford, U.K.;

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http://evolve.zoo.ox.ac.uk/). The region analyzed contained a total of 1076 bp. We deposited the alignment in TreeBase as submission No. 11139. Maximum parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford 2002) as heuristic searches with 1000 random addition replicates and TBR branch swapping. Support for the branching topologies was evaluated by bootstrap analysis derived from 1000 replicates with 10 random addition replicates each. Maximum likelihood (ML) analyses were conducted in RAxML-HPC2 7.2.7 (Stamatakis 2006) via the CIPRES portal (Miller et al. 2011) with default parameters adjusting for 1000 bootstrapping replicates and *Phyllotopsis nidulans* selected as outgroup.

Initial BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) queries of the LSU sequence of *C. brunneovelutina* indicated close affinities to *C. gruberi* and to members of genus *Cuphophyllus* (also known as *Camarophyllus*, which is an invalid name for this genus). A dataset was constructed from sequences (Moncalvo et al. 2000, 2002; Walther et al. 2005; Matheny et al. 2006, 2007; Garnica et al. 2007; Porter et al. 2008; Saar et al. 2009) downloaded from GenBank to include (i) representative members of genera *Cantharocybe* and *Cuphophyllus*, (ii) exemplars from other genera supported within the hygrophoroid clade based on the six-gene analyses of Binder et al. (2010) and Matheny et al. (2006), and (iii) outgroup taxa *Phyllotopsis nidulans* (Pers.) Singer and *Xeromphalina campanella* (Batsch) Maire also selected based on Matheny et al. (2006), Binder et al. (2010).

TAXONOMY

Cantharocybe was erected to accommodate a clitocyboid taxon with distinctive cheilocystidia and long, cylindrical, inamyloid spores. The new species described below, *C. brunneovelutina*, shares the clitocyboid habit, solid stipe and presence of cheilocystidia with the type species of *Cantharocybe*, *C. gruberi*, but it differs in having a velutinous, brown instead of a glabrous yellow pileus, elliptical instead of cylindrical spores, and in cystidial morphology. Because of these marked differences we think it necessary to emend the generic concept as well as describe a new species.

CANTHAROCYBE H.E. Bigelow & A.H. Smith, gen. emend., fide Smith (1944), Bigelow and Smith (1973).

Pileus 8–200 mm diam, convex-hemispheric to broadly convex with inrolled margin, surface dry, smooth or finely velutinous, partly areolate, margin not striate, pale yellow to lemon yellow overall or dark brown overall when young and over central area when mature, brownish gray at margin with age; context white, odor farinaceous, or variable, radish-like, somewhat sweetish or like green pepper, flavor mild or sweet and farinaceous.

Lamellae 4–8 mm wide, long decurrent or adnate with decurrent tooth, often anastomosing or forming a reticulum at the stipe apex, buff or concolorous with pileus edge concolorous, entire, a few forked or back-forked at margin and stipe; lamellulae in numerous tiers.

Stipe 30–95 mm long, 8–25 mm thick, slightly clavate, often tapered, surface dull, moist, glabrous or pruinose, concolorous with the pileus or brownish gray over lower half, light brownish gray to buff at apex, some areolate over lower one-third.

Spores 9–16 × 5.5–6 μm, elliptical or narrowly elliptical to oblong in profile and face view, often slightly tapered to hilar appendage end in both views, smooth, thin-walled, hyaline, inamyloid. Basidia (40–) 45–53 × 10–12 μm, clavate, four-sterigmate, hyaline, highly guttulate in KOH, often with a large clamp connection at base. Cheilocystidia of two types: (i) lecythiform but sometimes with a mucronate apex, basal portion clavate to ventricose and narrowing toward the base, upper portion extending into an elongated neck with or without a rounded capitulum; (ii) body clavate with 1–4 sterigmoid or prong-like apical (or rarely lateral) appendages, extending at oblique angles and frequently swollen or capitate at the apex, body clavate, elliptical, often constricted at midsection, appendages tapered, smooth, thin-walled, hyaline. Hyphae of lamellar trama parallel, becoming subregular toward the margin, with walls swelling slightly to 0.5–0.8 μm thick, appearing refractive in KOH, hyaline. Subhymenium ca. 15–20 μm deep, pseudoparenchymatous, composed of short refractive hyphae. Pileus surface of two types: (i) a cutis of appressed or slightly interwoven hyphae, smooth, thin-walled, pale yellow, slightly translucent-refractive; (ii) trichodermium with hyphal end segments or end cells vertical, angled or sometimes interwoven, end cells cylindrical, clavate, ventricose, ventricose-rostrate, apex rounded, occasionally with subapical constriction, smooth, thin-walled, with translucent, light brown contents. Pileus trama of interwoven, radially disposed hyphae, hyaline, and a few conducting hyphae. Stipe surface of two types: (i) hyphae appressed to slightly interwoven, smooth, thin-walled, hyaline, with scattered caulocystidia like those of the lamellar edge; (ii) over upper third with fertile basidia intermixed with cystidia like those on the lamellar edge, surface of lower portion mainly of interwoven hyphae with end cells or end segments similar to those of the pileus surface. Clamp connections present but not on all hyphal septa or at the base of every basidium.

Cantharocybe brunneovelutina Lodge, Ovrebo & Aime sp. nov. FIGS. 1, 2, 3–9

MycoBank MB518968, GenBank HM588721

FIGS. 1–2. Basidiomata, holotype, *Cantharocybe brunneovelutina*. 1. *C. brunneovelutina* photographed in situ, with the limestone karst showing on the forest floor. 2. *C. brunneovelutina* showing the somewhat radial arrangement of the pileus context where it split naturally, and showing a cut basidiome. Bar = 10 mm.

Etymology. Brunneus (L) = brown, velutinus (L) = velvety, referring to the brownish, velvety pileus surface.

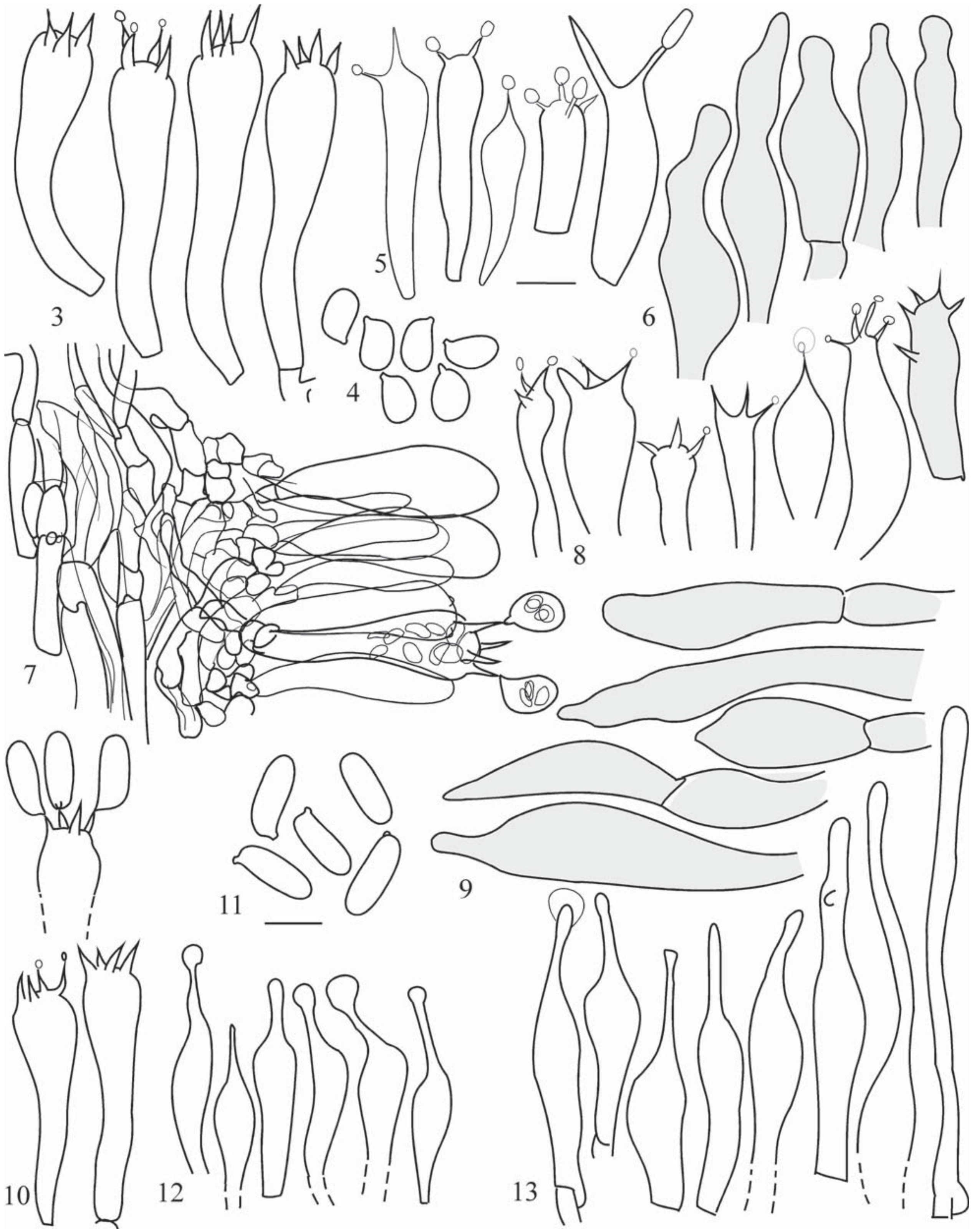
Pileus 55–75 mm diam, convexo-hemisphaericus, subvelutinus, atrobrunneus. Lamellae 4–8 mm latae, adnatae dente decurrenti praeditae, bubalinae. Stipes 58–95 mm longus, 8–18 mm crassus, subclavatus, pruinosus, brunneolo-cinereus. Sporae 9–9.5 × 5.5–6 μm, ellipticae, laeves, hyalinae, inamyloideae. Cheilocystidia appendiculis apicalibus spinul- vel spiculoideis saepe capitatis praedita. Superficies pilei pro parte maxima trichodermiata, hyphis translucentim brunneis. Superficies stipitis in parte inferiore pro parte majore implicata, hyphis translucentim brunneis. Caulocystidia ad stipitem sparsa, eis marginis lamellaris similia. Fibulae praesentes.

Pileus 55–75 mm diam, convex-hemispherical with inrolled margin, surface dry, finely velutinous, partly areolate, margin not striate, dark brown (Sepia to Hair Brown) overall when young and over central area when mature, brownish gray (Dark Drab to Drab) at margin with age; context white, odor farinaceous, flavor sweet and farinaceous.

Lamellae 4–8 mm wide, adnate with decurrent tooth, buff (pale horn to buff 124), edge concolorous, 1–2/mm at margin, 1/mm half way to margin, wavy, entire, a few forked or back-forked at margin and stipe; lamellulae in numerous tiers.

Stipe 58–95 mm long, 8–18 mm thick, slightly clavate, costate, 10–20 mm at apex, base often tapered, surface dull, moist, pruinose, brownish gray (Drab to Light Drab) over lower half, light brownish gray to buff (Drab Gray to Pale Horn) at apex, some areolate over lower one-third, solid, some caespitose.

Spores 9–9.5 × 5.5–6 μm (mean length = 9.15 μm, mean width = 5.83 μm, Q = 1.5–1.73, mean Q = 1.57), elliptical in profile and face view, often slightly tapered to hilar appendage end in both views, adaxial face occasionally flattened or slightly concave in profile, smooth, thin-walled, hyaline, inamyloid, acyanophilic. Basidia (40–)45–53 × (9–)10–11 μm, clavate, four-sterigmate, hyaline, highly guttulate in KOH, often with a large clamp connection at base. Lamellar edge heteromorphous. Cheilocystidia abundant, with 1–4 sterigmoid or prong-like apical (or rarely lateral) appendages, extending at oblique angles and frequently swollen or capitate at the apex, body 23–35 × 5–6 μm, clavate, elliptic, often constricted at midsection, smooth, thin-walled, hyaline, appendages 4–15 μm, tapered. Hyphae of lamellar trama 3–8 μm wide, parallel, becoming subregular toward the margin, with walls swelling slightly to 0.5–0.8 μm thick, appearing refractive in KOH, hyaline. Subhymenium ca. 15 μm deep, pseudoparenchymatous, composed of short refractive hyphae 3–4 μm wide. Pileus surface mostly a trichodermium with hyphal end segments or end cells vertical, angled or sometimes interwoven, layer 90–150 μm deep, end cells 45–80 × 9–16 (–20) μm, 10–20 μm wide, cylindrical, clavate, ventricose, ventricose-rostrate, apex rounded, occasionally with subapical constriction, smooth, thin-walled, with translucent, light brown contents. Pileus trama of interwoven but radially disposed hyphae 4–10(–12) μm wide, hyaline, and a few conducting hyphae 6.5–15 μm wide. Stipe surface over upper third with fertile basidia intermixed with cystidia like those on the lamellar edge; surface of lower portion mainly of interwoven hyphae with end cells or end segments similar to those of the pileus surface, end segments



FIGS. 3–13. Microscopic features of *Cantharocybe* species. 3–9. *C. brunneovelutina* (holotype). 3. Basidia. 4. Spores. 5. Cheilocystidia. 6. Hyphal end cells from stipe surface. 7. Lamellar trama subhymenium and hymenium. 8. Caulocystidia. 9. Pileus surface end cells. 10–13. *Cantharocybe gruberi* (holotype). 10. Basidia. 11. Spores. 12. Cheilocystidia. 13. Cystidia at stipe apex. Bar = 10 μm.

cylindrical, clavate, ventricose-rostrate, smooth, thin-walled, translucent light brown; the lower portion also with scattered caulocystidia with 1–5 prong-like appendages, body 25–38 × 6–10 µm, clavate or cylindrical, appendages 2–10 µm long, tapered, often with capitate tips, rarely with a secretion on the appendages, hyaline or with brownish translucent contents. Hyphae of stipe trama 4–10 µm wide, hyaline. Clamp connections present but not on all hyphal septa nor at the base of every basidium.

Holotype. BELIZE. ORANGE WALK DISTRICT: La Milpa Field State, La Milpa Archaeological Site, in humus around dead palm tree, 25 Oct 2002, DJL-BZ-85 (BZ-1883), 17°50′30″N, 89°1′0″W, 100 m (CFMR). Isotype BRH.

Commentary. Cantharocybe brunneovelutina is characterized macroscopically by the subvelutinous, brown pileus and pale brownish gray pruina on the stipe (FIGS. 1, 2) and microscopically by the unique cystidia that are present on the lamellar edge and stipe (FIGS. 5, 6, 8). Most cystidia somewhat resemble basidia in shape but the prong-like appendages at the apex are not sterigmata. These appendages number 1–5 and often extend at oblique angles. The cystidial appendages often have a small capitulum at the tip but these never develop into mature spores, and they do not appear to be secretory. However, there are rare secretory cystidia on the upper stipe surface (FIG. 8, third from right).

The type, *Cantharocybe gruberi* (A.H. Sm.) H.E. Bigelow and A.H. Sm., is the only other species in the genus. *Cantharocybe brunneovelutina* differs from *C. gruberi* in a number of ways: paler pileus and stipe pigmentation in *C. gruberi*; long-decurrent lamellae of *C. gruberi* but adnate with a subdecurrent tooth for C. *brunneovelutina*; shorter, broader spores in *C. brunneovelutina*; differences in pellis micro-anatomy; and different cheilocystidia morphology. The pileus pigment is dark brown for *C. brunneovelutina* from an intracellular brown pigment, whereas the pileus is yellow in *C. gruberi*; the pileus surface of *C. brunneovelutina* is mainly a trichodermium arising from a somewhat interwoven layer, whereas it is a an interwoven cutis with pale yellow hyphae in *C. gruberi*; the cheilocystidia of *C. brunneovelutina* most often have multiple prong-like appendages with or without an abruptly rounded capitulum that almost resembles a developing spore, those of *C. gruberi* are long-mucronate with or without a capitate apex that is more gradually enlarged (lecythiform).

Based on the morphological differences, one might not be easily led to conclude that the Belizean fungus is a *Cantharocybe*. Both species however share these features: robust basidiomes with solid stipes; a deep pseudoparenchymatous subhymenium of short, refractive, interwoven hyphae; mostly regular lamellar context composed of cylindrical hyphae with walls often slightly thickened and refractive when viewed in KOH; large but inconstant clamp connections on the hyphae and basidia bases; inamyloid context and spores; capitate cystidia and infrequent fusoid or lecithiform caulocystidia with secretions on the apex.

Type study of Cantharocybe gruberi.—Our recent study of the holotype was used to compare the micromorphology with *C. brunneovelutina* because *C. gruberi* has been rarely reported in the literature. We also studied one of the *C. gruberi* collections cited in GenBank (PBM 510) and confirm that its morphology agrees with the holotype.

Cantharocybe gruberi (A.H. Sm.) H.E. Bigelow & A.H. Sm., Mycologia 65:485. 1973. FIGS. 10–13

≡ *Clitocybe gruberi* A.H. Sm., Mycologia 36:245. 1944

≡ *Laccaria gruberi* (A.H. Sm.) Singer, Lilloa 22:176. (1949) 1951.

Spores (13.5)14–15(16) × 5.5–6 µm (mean = 14.73 × 5.63 µm, Q = 2.33–2.8, mean Q = 2.63), narrowly elliptic to oblong in profile and face view, smooth, thin-walled, hyaline, inamyloid. Basidia 40–50 × 10–12 µm, four-sterigmate, clavate, hyaline. Cheilocystidia present on both the main lamellae and also on the lamellar ridges/reticulum that extend onto the stipe; those on the main lamellae approximately lecythiform but sometimes with a mucronate apex, basal portion clavate to ventricose and narrowing toward the base, upper portion extending into an elongated neck with or without a rounded capitulum, entire length 40–47 µm, neck 10–20 × 2–3 µm, capitulum to 4 µm wide, smooth, thin-walled, hyaline; those on lamellar ridges of stipe of a similar shape but much more numerous and almost forming a continuous layer, often with a longer apex, entire length 40–60 µm, 6–10 µm where widest, apex 8–43 µm long and 2–3 µm wide, mainly capitate, some cystidia cylindrical and appearing filamentous, 60–85 × 3–3.5 µm, cystidia rarely with a secretion. Hyphae of lamellar trama parallel, 3–8 µm wide, cyclindrical, hyaline, with slightly thickened walls when viewed in KOH, the hyphae near the lamellar edge are narrow and resemble the subhymenial hyphae. Subhymenium a layer of interwoven hyphae 10–20 µm wide, hyphae 2.5–3.5 µm wide, cylindrical, slightly refractive, hyaline. Hyphae of pileus surface a cutis of appressed or slightly interwoven hyphae, 4–8 µm wide, smooth, thin-walled, pale yellow, slightly translucent-refractive; pileocystidia not seen. Hyphae of pileus trama to 10 µm wide, hyaline. Hyphae of stipe surface 3–5 µm wide, appressed to slightly interwoven, smooth, thin-walled, hyaline, with scattered caulocystidia like those of the lamellar edge.

Hyphae of stipe trama up to 12 µm wide, hyaline. Clamp connections present but not at every cross wall.

Holotype collection. IDAHO: Jul, May 1943, W.B. Gruber 26 (MICH).

Commentary. Clitocybe gruberi was described from a single basidioma from Idaho (Smith 1944). In Smith's discussion of the species he commented that ''the long cylindric spores, the color of the carpophore [pileus pale yellow], and the cheilocystidia are an unusual combination of characters in *Clitocybe*.'' After studying more collections from New Mexico Bigelow and Smith (1973) erected *Cantharocybe* to accommodate this species that they concluded was not a *Clitocybe*, their justification formulated from the above observations made by Smith. The Bigelow and Smith study also provided an updated description of the species that included illustrations of spores and cheilocystidia, but they did not indicate whether the illustrations were from the type or supplemental material. We provide here detailed illustrations from the holotype (FIGS. 10–13).

With two exceptions our study of the type material agrees with the protolog. Smith in the protolog mentioned for the pileus surface that ''hyphal tips present as pilocystidia, the end cell not differentiated except for a narrow, short proliferation, the cells 20–35 × 4–7 µm, proliferation 10–15 µm long and 2–4 at the base …''. We could not confirm the presence of such structures in our examination of the type, in our examination of supplemental collections, and these pileocystidia were not mentioned in the redescription of *C. gruberi* by Bigelow and Smith (1973). Second, Smith stated that clamp connections were absent, but we have confirmed their presence, as did Bigelow and Smith in their later description.

Supplemental material studied. CALIFORNIA: Sierra County: Green Acres, Basset's Junction, Yuba Pass, beneath *Abies* and *Pinus*, 3 Jun 1997, *PBM 510*. IDAHO: Valley County: Cascade Christian Camp, on soil, mixed conifer woods, 15 Jun 1986, coll. *J. Olson, JFA 9307*. WASHINGTON: Chleland County: Tumwater Campground area, H.W. 2 across from C.G., soil, mixed woods, 26 May 1991, coll. *H. Hendrickson, JFA 10256*. Klickatat County: Gifford Pinchot National Forest, Peterson Prairie Road out of Trout Lake, 4 Jun 1988, coll. *Jan Lindgren, LLN 92.04.13-3*. Okanogan County: Okanogan National Forest, Mount Annie, F.S. Road 300, 25 Jun 1997, solitary on conifer stump, *MTS 4391* (all at WTU).

MOLECULAR PHYLOGENETIC RESULTS

Our analyses of the LSU region show *C. brunneovelutina* and *C. gruberi* as strongly supported sister taxa (FIG. 14). *Cantharocybe* and *Cuphophyllus* appear closely related in these and other analyses (not shown), although without strong support. This placement is consistent with the placement of *C. gruberi* in the hygrophoroid clade near *Cuphophyllus* (as *Camarophyllus*) and *Ampulloclitocybe* based on six gene regions (Binder et al. 2010).

The placement of *Cantharocybe* within the Agaricomycetes has not been resolved satisfactorily. Singer (1951) originally placed *Clitocybe gruberi* in *Laccaria* Berk. & Broome, but Singer (1986) admitted that it was not a *Laccaria* and included it under the heading of ''Genera Incompletely Known'' at the end of the section on the Paxillaceae. He thought that the genus could be inserted between *Omphalotus* Fayod and *Hygrophoropsis* (J. Schröt.) Maire ex Martin-Sans of the Paxillaceae. Molecular analyses based on six gene regions (Binder et al. 2010) weakly support *Cantharocybe* within the Hygrophoraceae near *Ampulloclitocybe clavipes* and *Cuphophyllus* (as *Camarophyllus*). In Binder et al. (2010) the type species of *Cantharocybe* and *Ampulloclitocybe* appear together on long branches within the same clade with less than 50% bootstrap support while the subtending branch supporting *Cantharocybe* and *C. basidiosus* (as *Camarophyllus basidiosus* because this species has not yet been recombined in *Cuphophyllus*) has a Bayesian posterior probability of 1.0. In our analyses of the LSU region *C. brunneovelutina* and *C. gruberi* appear together on a strongly supported branch while the subtending branch joining *Cantharocybe* and *Cuphophyllus* is only weakly supported (FIG. 14).

DISCUSSION

Cantharocybe is placed among the basal genera of the hygrophoroid clade (i.e. Hygrophoraceae) based on molecular analyses with low support (Binder et al. 2010, Lodge et al. unpubl). It can be distinguished from related genera, except *Xeromphalina*, based on the presence of cheilocystidia. *Xeromphalina* differs from *Cantharocybe* in having amyloid spores and a marasmioid or collybioid appearance. In a recent four-gene analysis (Lodge et al. unpubl) *Cantharocybe* and *Ampulloclitocybe* form a separate clade with 0.98 Bayesian support but low bootstrap support (36–70%) among genera of the Tricholomataceae near the base of the Hygrophoraceae. Due to weak support values in both analyses, the family to which *Cantharocybe* should be placed remains uncertain. The macroscopic and some microscopic features of *C. brunneovelutina* most closely resemble those of *Ampulloclitocybe clavipes* and *Cuphophyllus* species—a placement supported by some of the molecular analyses. The cylindrical context hyphae and slightly thickened (0.5–0.8 µm) and refractive walls that are found in both species of *Cantharocybe* are characteristic of most species of *Cuphophyllus* as well as *Ampulloclitocybe. Ampulloclitocybe clavipes* and *C. brunneovelutina* both have trichodermial pileipellis elements with dissolved

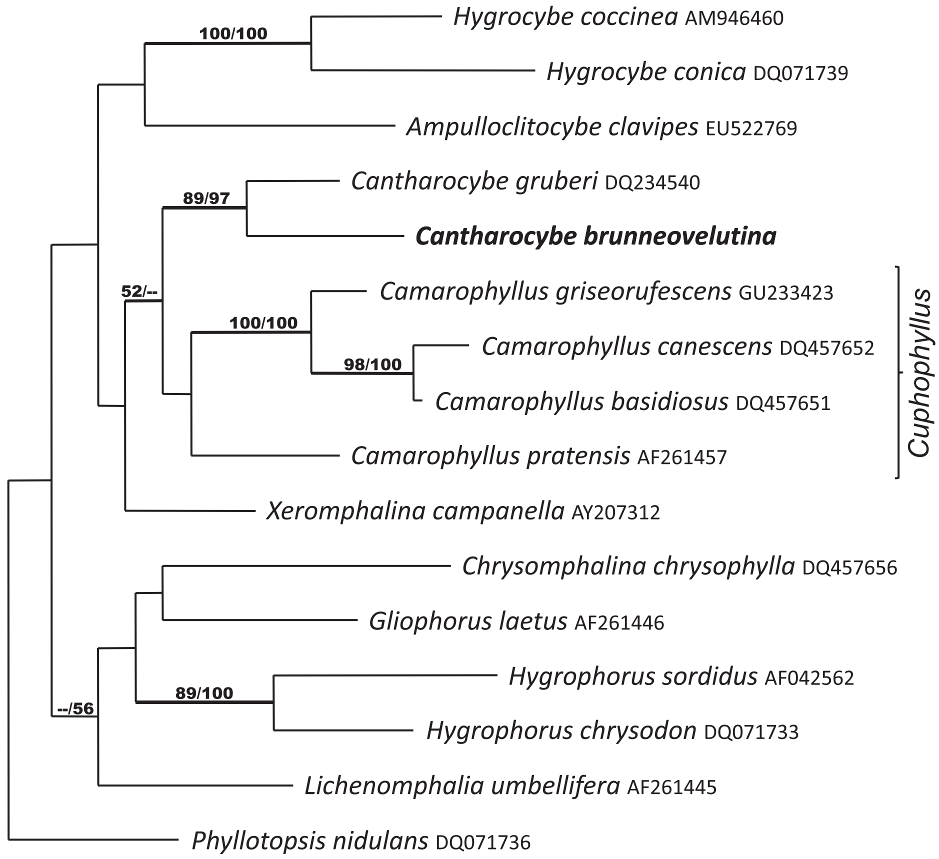


FIG. 14. One of four equally parsimonious trees resulting from phylogenetic analyses of LSU rDNA sequences of 16 taxa of Hygrophoraceae. Numbers above branches are MP bootstrap (1000 replicates) values followed by ML bootstrapping (1000 replicates) values. Branches receiving greater than 50% bootstrap support that are topologically congruent with ML analyses are indicated in boldface. Note that GenBank accession names are used for relevant taxa. Although *Camarophyllus* is an invalid genus name for *Cuphophyllus*, most GenBank entries of these taxa are accessioned under *Camarophyllus*.

brown pigments, but *Ampulloclitocybe* lacks cheilocystidia. While the robust basidiomes, solid stipe and decurrent or adnate-decurrent lamellae are shared with *Cuphophyllus*, the lamellar trama in *Cantharocybe* is regular whereas it is interwoven in typical members of *Cuphophyllus* as well as *Ampulloclitocybe*. Singer (1986) noted some exceptions in *Cuphophyllus* (as *Camarophyllus*) in which the lamellar trama was predominantly regular, but the species to which he referred were moved to *Camarophyllopsis*, a genus now placed outside the Hygrophoraceae and close to the base of the Agaricales (Matheny et al. 2006, Binder et al. 2010). However the presence of cheilocystidia and ornamented caulocystidia clearly sets *Cantharocybe* apart from *Cuphophyllus*, a distinction supported in molecular analyses (in the present paper and in Binder et al. 2010) that place these genera in separate clades.

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