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THE ENTOLOMATACEAE OF THE PAKARAIMA MOUNTAINS OF GUYANA VII: *ENTOLOMA DICYMBOPHILUM* SP. NOV. AND A KEY TO GUYANESE SPECIES OF *ENTOLOMA S. STR.*

T. W. Henkel* & D. L. Largent

Entoloma dicymbophilum sp. nov. is described from the Pakaraima Mountains of Guyana. The species is unique within the genus in its combination of large, white, tricholomatoid basidiomata with isodiametric, angled basidiospores. A key to species of *Entoloma s. str.* from Guyana is provided.

Keywords. Agaricales, Basidiomycota, *Dicymbe*, Guiana Shield, Neotropical fungi
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Introduction

The Entolomataceae (Agaricales, Agaricomycetes, Basidiomycota) is a large family of > 1500 species of mostly lamellate mushrooms occurring worldwide in temperate and tropical forests (Noordeloos & Gates, 2012). Fungi in this group were originally classified by Fries in 1838 in *Agaricus* tribe *Entoloma* Fr. and subsequently raised to the family level by Kotlaba & Pouzar (1972). Genera within the Entolomataceae have traditionally been viewed as one large genus, *Entoloma* (Fr.) P.Kumm., with recognition of multiple subgenera (e.g. Noordeloos, 1992), or as a narrow *Entoloma s. str.* plus multiple segregate genera (e.g. Pegler, 1977; Largent, 1994).

Many saprotrophic Entolomataceae species with collybioid or clitocyboid statures and angled heterodiametric basidiospores occur in tropical rain forests worldwide (e.g. Horak, 1978; Largent *et al.*, 2020; Reschke *et al.*, 2022). More rarely encountered in the tropics are species with tricholomatoid statures and angled isodiametric basidiospores (e.g. Romagnesi & Giles, 1979; Largent *et al.*, 2008). Fungi with the aforementioned features would be placed in *Entoloma s. str. sensu* Largent (1994) or *Entoloma* subg. *Entoloma sensu* Noordeloos (1981) and are treated as putatively ectomycorrhizal (ECM) (e.g. Zerova & Rozhenko, 1966; Antibus *et al.*, 1981; Montecchio *et al.*, 2006).

Over 20 years of collecting in remote Neotropical rain forests of the Pakaraima Mountains of Guyana, in the central Guiana Shield region, has yielded a plethora of new macrofungal species and genera. In the Entolomataceae, numerous new species have been described in the saprotrophic genera *Alboleptonia* Largent & R.G.Benedict, *Calliderma* (Romagn.) Largent, *Inocephalus* (Noordel.) P.D.Orton, *Nolanea* (Fr.) P.Kumm., *Paraeccilia*

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Largent, *Rhodocybe* Maire and *Trichopilus* (Romagn.) P.D.Orton (Largent *et al.*, 2008; Aime *et al.*, 2010; Henkel *et al.*, 2010a, 2010b, 2014). However, only four species have currently been described in *Entoloma s. str.* from forests dominated by ECM trees of the leguminous genera *Dicymbe* Spruce ex Benth. and *Aldina* Endl. (Largent *et al.*, 2008).

Here, we describe a new species of *Entoloma s. str.* (= *Entoloma* subg. *Entoloma sensu* Noordeloos, 1992) based on its tricholomatoid stature, glabrous or innately fibrillose pileus surface, a stipe apex that is > 4 mm broad, sinuate lamellae, subisodiametric to isodiametric basidiospores, broad pileus trama hyphae, minimal oleiferous hyphae, and abundant clamp connections (Largent, 1994). Sequence data are provided from the holotype for the ITS and 28S loci.

Methods and materials

The collection was made during the June 2013 rainy season in Guyana's Pakaraima Mountains 2 km S of the Potaro River near Tadang base camp located at 5°16'14.5"N, 59°50'39.1"W, 710–750 m, in a forest codominated by the ECM trees *Dicymbe corymbosa* Spruce ex Benth. and *Dicymbe altsonii* Sandwith (Smith *et al.*, 2011). Methods for field description, microscopic analyses, and image capture followed Largent *et al.* (2008, 2020). Fungi were field-dried with silica gel. Colour designations follow Kornerup & Wanscher (1978), with colour plates noted in parentheses (e.g. 4A7). The type specimen was deposited in BRG (holotype) and HSC-F (isotype) (herbarium codes follow Thiers, continuously updated). Dried basidiomata were sectioned and rehydrated in 3% KOH.

A Nikon Eclipse Ci compound microscope (Nikon, Tokyo, Japan) with Lumenera Infinity 2 imaging software (Teledyne Lumenera, Ottawa, ON, Canada) was used to measure microscopic features following Largent (1994) and capture photomicrographs following Largent *et al.* (2020). In the taxonomic description, $\underline{x}$ refers to mean dimensions of structures ± standard deviations, *E* refers to range of length of structures divided by their width, *Q* refers to the mean quotient of the length of structures divided by their width, and *n* refers to the number of objects measured.

DNA was extracted from fungal tissue by heating with a Chelex extraction buffer (100 mM Tris pH = 8.5, 4% Chelex 100, 1% Triton X-100) at 99°C for 20 min, then freezing. After thawing, the resulting supernatant was used in PCR.

The PCR was performed in 25 mL reactions with 1 µL of DNA extract, 0.4 mM each primer, 0.2 mM dNTP mixture, 5 µg of bovine serum albumin, and 0.5 U of OneTaq Hot Start DNA polymerase (New England Biolabs, Ipswich, MA, USA) in 1X OneTaq standard buffer. PCR conditions were 94°C for 30 s, followed by 36 cycles of 94°C for 15 s, 57°C for 30 s and 68°C for 60 s, followed by a final extension at 68°C for 5 min. Primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990) were used to amplify the ITS1–5.8S–ITS2 region.

Sequencing was performed at Eurofins Genomics (Louisville, KY, USA) using the same primers used for PCR. Forward and reverse reads were aligned using Genious 10.2.4

(https://www.geneious.com) and resolved manually, as needed. BLAST searches for the sequences obtained were carried out on GenBank.

Results

One ITS (GenBank accession no. OR168649) sequence and one 28S rRNA (GenBank accession no. OR146595) sequence were generated from the type collection in the present study. The BLAST searches on GenBank for both sequences revealed affinity of the new species with the Entolomataceae but low percent similarities with other species within the family for which sequence information was available.

Species description

Entoloma dicymbophilum Largent & T.W.Henkel, **sp. nov.**

Similar to *Entoloma fragilum* Largent & Aime in its white colour but differs in its larger fleshy-fibrous basidiomata, larger basidiospores, longer basidia, and musky, clay-like odour. – Type: Guyana, Region 8 Potaro-Siparuni, Pakaraima Mountains, Upper Potaro River Basin, 2 km S of Potaro River at Tadang base camp at 5°16'14.5"N, 59°50'39.1"W, 710–750 m, on humic mat of forest floor under *Dicymbe corymbosa* and *D. altsonii*, 4 vi 2013, *T. Henkel* 9740 (holotype BRG, isotype HSC-F [G1429]). MycoBank MB844000. GenBank accession nos.: ITS = OR168649; 28S = OR146595. **Figures 1, 2.**

Basidiomata tricholomatoid. *Pileus* 48–110 mm broad, 11–20 mm tall, initially broadly conic with downturned, irregularly sulcate margin, with age broadly convex to uplifted with broad, rounded umbo, off-white to pale cream (4A2–4A3) throughout, glabrous, moist; context off-white, unchanging, 8–10 mm thick above stipe. *Lamellae* thick, subdistant, broadly sinuate with distinct decurrent teeth, initially light creamish tan with faint pinkish tones (4A3–5A3), with age dull pink, 2 mm tall at margin, 4–6 mm centrally, 3–4 mm at stipe; edges concolorous, smooth, unchanging; lamellulae 1–3, 5–30 mm long. *Stipe* 95–150 × 9–20 mm, subequal, enlarging slightly and evenly towards base, curving and occasionally twisting, solid, off-white to pale orangish cream (4A3–4A4) throughout, shallowly longitudinally striate, apical 1/3 with scattered minute orangish squamules visible under hand lens; basal mycelium a dense white mat surrounding base; context off-white, unchanging, densely fibrous throughout. *Odour* somewhat musky, clay-like. *Taste* faintly acrid-astringent, slightly bitter.

Basidiospores subisodiametric to isodiametric, 6-angled; angles initially smooth and indistinct, becoming distinct with maturation; apex very slightly acute; contents multiglobular or without globules, rarely with a single globule, 6.9–8.9 × 6.2–8.6 µm ($\underline{x}$ = 7.9 ± 0.4 × 7.5 ± 0.4 µm; *E* = 1.0–1.2; *Q* = 1.1 ± 0.04; *n* = 75). *Basidia* narrowly cylindro-clavate and tapered to a narrow base, with granules at maturity, 43.1–65.1 × 6.9–10.9 µm ($\underline{x}$ = 52.9 ± 5.8 × 9.1 ± 1.1 µm; *E* = 4.6–8.5; *Q* = 5.9 ± 1.1; *n* = 31), 2 or 4 sterigmate; sterigmata up to

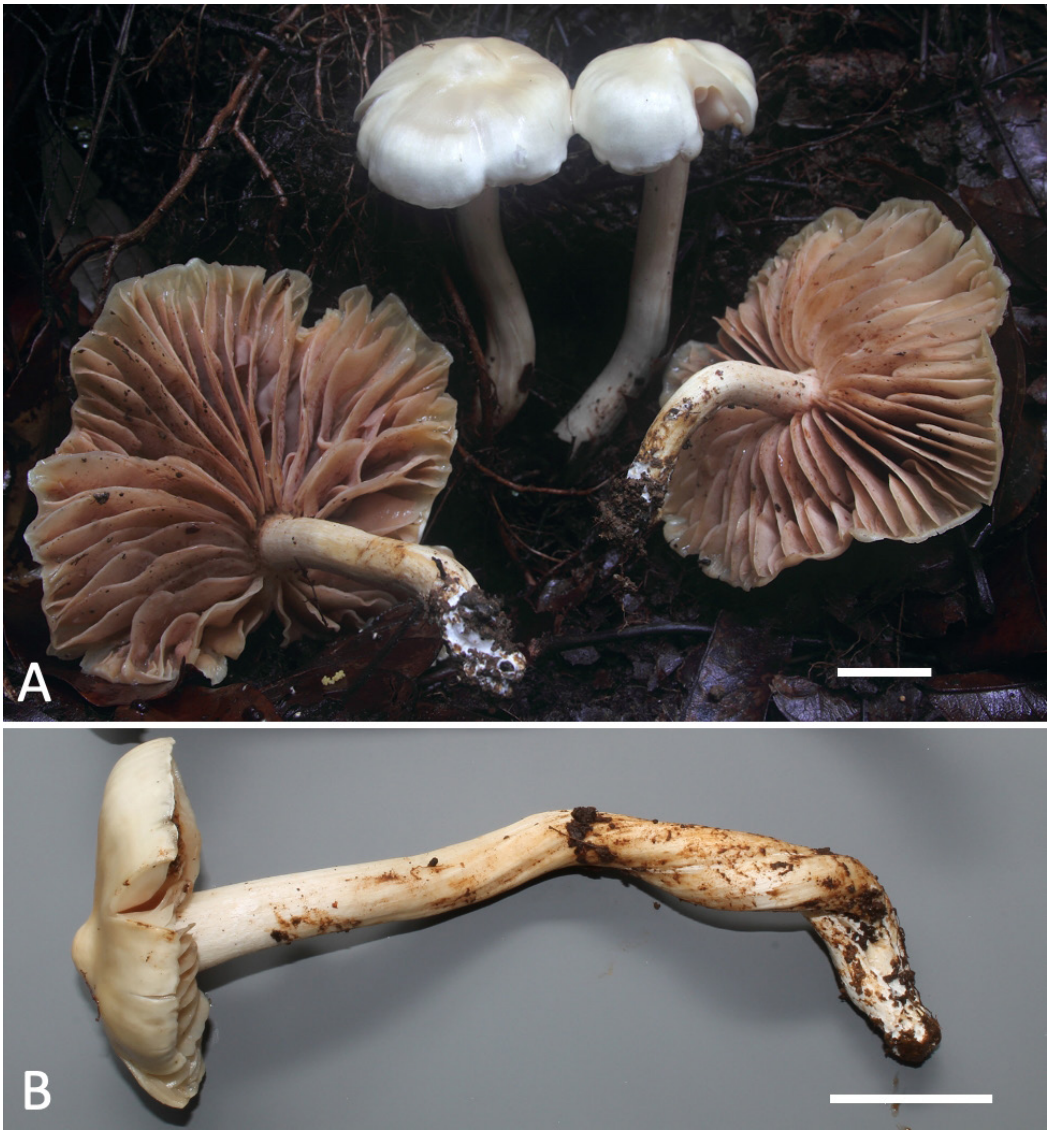

Figure 1. *Entoloma dicymbophilum* Largent & T.W.Henkel, sp. nov. A, Field habit, off-white to cream basidiomata with exposed lamellae, Upper Potaro Basin, Guyana; B, immature basidioma (lateral view). Each scale bar, 20 mm. Photographs of the holotype, *Henkel* 9740, taken by Todd Elliott (A) and Terry Henkel (B).

6.9 µm long (*n* = 21). *Cheilocystidia* and *pleurocystidia* absent. *Lamellar trama* 152–276 µm wide; hyphae subparallel to interwoven; cells long and narrow, 27.0–79.2 × 3.4–9.7 µm ($\underline{x}$ = 51.6 ± 13.7 × 6.7 ± 1.4 µm; *E* = 3.0–18.01; *Q* = 8.2 ± 3.1; *n* = 33). *Pileipellis* in radial section 27–65 µm thick, of tightly entangled, subpericlinal hyphae, these 3–5 µm wide; pileocystidia

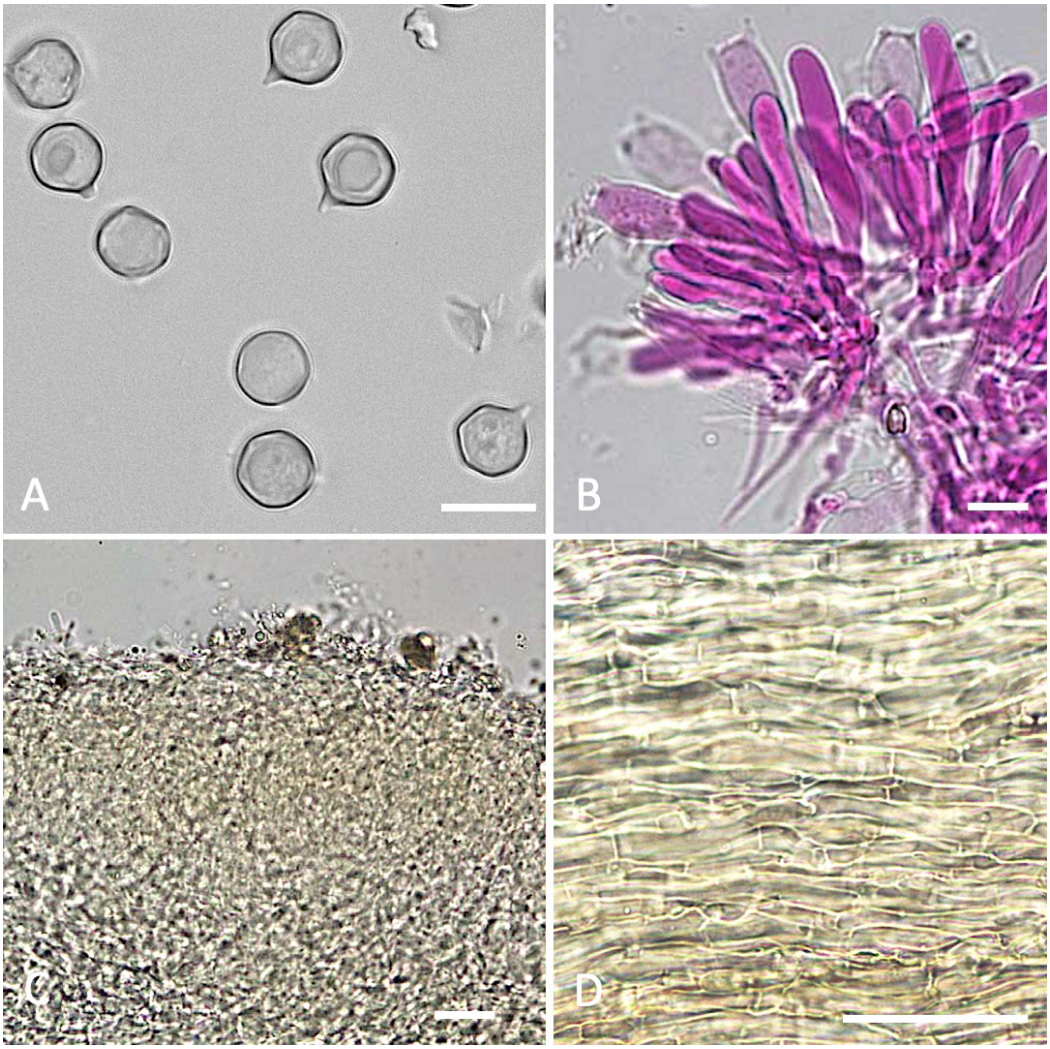

Figure 2. Microscopic features of *Entoloma dicymbophilum* Largent & T.W.Henkel, sp. nov. A, basidiospores; B, basidia and basidioles; C, pileipellis and subtending trama; D, pileus trama. All photographs of the holotype, *Henkel* 9740, taken by D. L. Largent. Scale bars: A and B, 10 µm; C and D, 50 µm.

scattered, suberect, cylindrical, 7.0–16.0 × 1.5–4.2 µm ($\underline{x}$ = 11.4 ± 2.9 × 2.9 ± 0.8 µm; *E* = 2.7–5.5; *Q* = 4.1 ± 0.9; *n* = 14). *Pileus trama hyphae* in squash mounts relatively short and broad, 16.0–74.1 × 2.8–16.7 µm ($\underline{x}$ = 35.9 ± 13.4 × 8.6 ± 3.1 µm; *E* = 1.6–10.8; *Q* = 4.6 ± 2.1; *n* = 66). *Stipitipellis* at the stipe apex composed of scattered hymeniform clusters lacking basidia, at times with semi-erect and scattered caulocystidia, these cylindrical, rarely clavate, 10.5–21.0 × 3.0–7.0 µm ($\underline{x}$ = 15.0 ± 4.0 × 4.0 ± 2.0 µm; *E* = 3–5; *Q* = 4.0 ± 1.1; *n* =

5). *Stipe trama hyphae* subparallel and entangled in longitudinal sections; cells 19.8–97.8 × 2.9–10.3 µm ($\underline{x}$ = 47.1 ± 18.6 × 6.4 ± 1.7 µm; *E* = 2.5–14.7; *Q* = 7.5 ± 2.7; *n* = 57). *Oleiferous hyphae* very rare in the trama of the pileus, stipe and lamellae. *Lipoidal globules* absent. *Clamp connections* evident and abundant in hyphae of the subhymenium, pileus trama, lamellae and stipe.

Distribution. Known only from the type locality in the Upper Potaro River Basin of Guyana.

Habitat and ecology. In pairs on humic mat of forest floor under *Dicymbe corymbosa* and *D. altsonii*.

Etymology. *Dicymbe* and *-philus* (Greek, 'loving'), referring to the occurrence in forests with *D. corymbosa* and *D. altsonii*.

Proposed IUCN conservation category. Data deficient (DD) due to inadequate information for the species' geographical distribution or population status in this remote, mycologically underexplored region.

Notes. *Entoloma dicymbophilum* is recognised in the field by its medium to large, entirely off-white to pale-cream basidiomata with solid trama, smooth, moist pileus, thick, subdistant, sinuate, off-white to dull-pink lamellae, and clay-like musky odour. The species fits well into *Entoloma s. str.* based on its robust, tricholomatoid stature, isodiametric 6-angled basidiospores, narrowly cylindro-clavate basidia, broad pileus tramal hyphae, and abundant clamp connections (Largent, 1994). The species is putatively ECM based on its inclusion in *Entoloma s. str.* and occurrence in forests dominated by ECM *Dicymbe* trees (Largent *et al.*, 2008).

Entoloma fragilum is another white-coloured Guyanese species with isodiametric basidiospores, but it differs from *E. dicymbophilum* in its smaller, brittle basidiomata, smaller basidiospores (4.9–6.8 × 4.8–6.7 vs 6.9–8.9 × 6.2–8.6 µm), shorter basidia (28.2–36.2 vs 43.1–65.1 µm), and pleasant, *Lactarius*-like odour (Largent *et al.*, 2008). The other sympatric *Entoloma s. str.* species in Guyana, *E. olivaceocoloratum* Largent & T.W.Henkel, *E. rugosostriatum* Largent & T.W.Henkel and *E. illinitum* Largent & Aime, all differ from *E. dicymbophilum* in their much darker olive-brown, greyish brown, or yellowish brown pileus colours, respectively, among other features (Largent *et al.*, 2008).

Several other tropical Entolomataceae species are known with tricholomatoid statures and entirely white basidiomata. These include *Entoloma leviculum* Corner & Horak from Sabah, which can be differentiated from *E. dicymbophilum* by its lack of odour and smaller overall size, with the pileus 15–25 mm broad and the stipe 30–50 × 3–5 mm (Horak, 1980). The Antillean *Entoloma cerrusatum* Pegler, *E. bakeri* Dennis (Pegler, 1983), Malagasy *E. vetulum* (Romagn.) Noordel. & Co-David (Romagnesi, 1941), and West African *E. candicans* (Romagn. & Gilles) Noordel. & Co-David and *E. eburneum* (Romagn. & Gilles) Noordel. & Co-David (Romagnesi & Giles, 1979) are white and tricholomatoid but each

differ from *E. dicymbophilum* in their heterodiametric basidiospores that are > 8.5 µm long. The Cameroonian *Entoloma intricatum* Largent, T.W.Henkel & R.A.Koch has large, off-white basidiomata but differs from *E. dicymbophilum* in its naucorioid stature with adnate lamella attachment and strongly heterodiametric, 5- to 7-angled basidiospores (Largent *et al.*, 2020). Among white Neotropical *Alboleptonia* species described from Guyana or Panama, all differ from *Entoloma dicymbophilum* in their slender, collybioid or clitocyboid statures and heterodiametric basidiospores (Henkel *et al.*, 2010b; Reschke *et al.*, 2022). Brazilian *Entoloma* species with isodiametric basidiospores similar to those of *E. dicymbophilum* differ in numerous other macro- and micromorphological features (Coimbra *et al.*, 2013).

Key to species of *Entoloma s. str.* known from the Pakaraima Mountains of Guyana

1a. Pileus off-white to pale cream–coloured _____ 2
1b. Pileus olive-brown, greyish brown, or yellowish brown _____ 3

2a. Pileus 50–62 mm broad, dry; odour fragrant, pleasant, *Lactarius*-like _____ *E. fragilum*
2b. Pileus 48–110 mm broad, moist; odour unpleasant, musky, clay-like ___ **E. dicymbophilum**

3a. Pileus yellowish brown; odour radish-like _____ *E. illinitum*
3b. Pileus olive-brown or greyish brown; odour mild, not radish-like _____ 4

4a. Pileus olive-brown, > 90 mm in diameter; margin not striate _____ *E. olivaceocoloratum*
4b. Pileus greyish brown, < 50 mm in diameter; margin striate _____ *E. rugosostriatum*

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