

A new species of *Clandestinotrema* (Ascomycota: Ostropales: Graphidaceae) from montane cloud forest in the Venezuelan Andes

Ian D. Medeiros

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Abstract. Revision of lichen herbarium specimens at DUKE revealed a species of thelotremoid *Graphidaceae* new to science. *Clandestinotrema carbonera* sp. nov. is characterized by an olive-green thallus, dense cortex, the absence of a columella, ascospores $15\text{--}24 \times 5.5\text{--}8.0 \mu\text{m}$ with 3–5 transverse septa (occasionally submuriform), and the presence of hypostictic acid as a major substance. At present the species is known only from the type locality, an area of montane cloud forest near Mérida, Venezuela. Interestingly, this species appears to have been discussed in the literature once before, as an unnamed ‘*Thelotrema* sp.’ which in the 1970s provided the first instance of hypostictic acid isolated from a lichen. The previous report of *C. leucomelaenum* from the same locality is shown to actually represent *C. pauperius*.

Key words: El Bosque San Eusebio-La Carbonera, *Fissurinoideae*, *Lecanoromycetes*, Neotropics, taxonomy, tryptethelioid ascospores

Introduction

In the last fifteen years, extensive revision of the family *Graphidaceae* (e.g., Staiger 2002; Frisch et al. 2006; Lücking et al. 2009; Rivas Plata et al. 2010, 2012) has made possible the recognition and description of hundreds of species new to science (Lücking et al. 2014). Many of these new discoveries came about via the reevaluation of existing herbarium material; for example, Lücking (2015) described 23 new species of thelotremoid *Graphidaceae* (species which previously would have been classified in the formerly independent family *Thelotremataceae*) from collections housed at the New York Botanical Garden Herbarium (NY). *Graphidaceae* is the largest family of crustose lichenized fungi in the tropics, and its actual diversity is predicted to be substantially larger than the number of species currently recognized (Lücking et al. 2014). The present paper contributes to our knowledge of the biodiversity of neotropical *Graphidaceae* with the description of a new species in the genus *Clandestinotrema*. This species is described from a previously unidentified specimen held in the Duke University Herbarium (DUKE). In addition, the previous report of *C. leucomelaenum* from the type locality of the new species is reassessed.

Materials and methods

Specimens were examined under a Leica MZ6 dissecting microscope. Ascomatal sections were cut by hand and mounted in tap water; observations and measurements of ascomata and spores were made with a Leica DMLB compound microscope at $400\times$. Photographs were taken with a Canon Rebel XSi camera attached to a Leica MZ125 dissecting microscope or a Zeiss Axioplan 2 compound microscope.

Secondary chemistry was assessed by thin-layer chromatography using standard methods (Culberson & Kristinsson 1970; Orange et al. 2010). Solvent system C (170 parts toluene: 30 parts glacial acetic acid) was used for the analysis. Spore and hymenium amyloidity was assessed with Lugol’s solution.

Results and discussion

Clandestinotrema carbonera I. Medeiros, sp. nov.

(Fig. 1)

MycoBank MB 825106

Diagnosis: Differing from *Clandestinotrema antoninii* in the olive-green thallus, smaller ascospores, and hypostictic acid as the major secondary metabolite.

Type: Venezuela, Mérida, La Carbonera cloud forest, s.d., M.F. Keogh 77211 (DUKE 0334483 – holotype).

Department of Biology, Duke University, Campus Box 90338, Durham, NC 27708, USA
Corresponding author e-mail: ian.medeiros@duke.edu

Description. Thallus corticolous, epiperidermal; surface smooth, olive-green; prothallus absent; thallus in section 40–100 μm thick, with dense, prosoplectenchymatous cortex 10–30 μm thick, irregular photobiont layer 30–75 μm thick, and indistinct medulla; lacking crystals. Photobiont *Trentepohlia*. Ascomata rounded, immersed, with complete thalline margin, 0.3–0.4 mm in diam. Disc covered by round pore 0.03–0.06 mm wide. Proper margin entire, not visible externally. Thalline margin entire, smooth, concolorous with thallus but with narrow pale zone around pore. Excipulum entire, prosoplectenchymatous, upper half carbonized, lower portion yellow-brown, 20–35 μm wide, divided from thalline margin by layer of periderm from substrate; completely covered by algiferous, corticate thallus 40–50 μm thick.

Columella absent. Hypothecium prosoplectenchymatous, 15–20 μm high, hyaline. Hymenium 75–90 μm high, hyaline, clear; epithecium indistinct, 4–6 μm high, gray. Paraphyses unbranched, apically smooth; periphysoids absent. Asci clavate, 75–90 \times 10–15 μm . Ascospores 8 per ascus, \pm uniseriate, ellipsoid, with 3–5 transverse septa, occasionally with single longitudinal septum in one of the central lumina, 15–24 \times 5.5–8.0 μm , 2–3 times as long as wide, hyaline, distoseptate with thick septa and diamond-shaped lumina, I–. Conidiomata not seen.

Chemistry. Hypostictic (major) and stictic (minor) acids confirmed with TLC. An annotation by Chicita Culberson also reports trace hyposalazinic and cryptostictic acids, but these were not verified in the new TLC analysis.

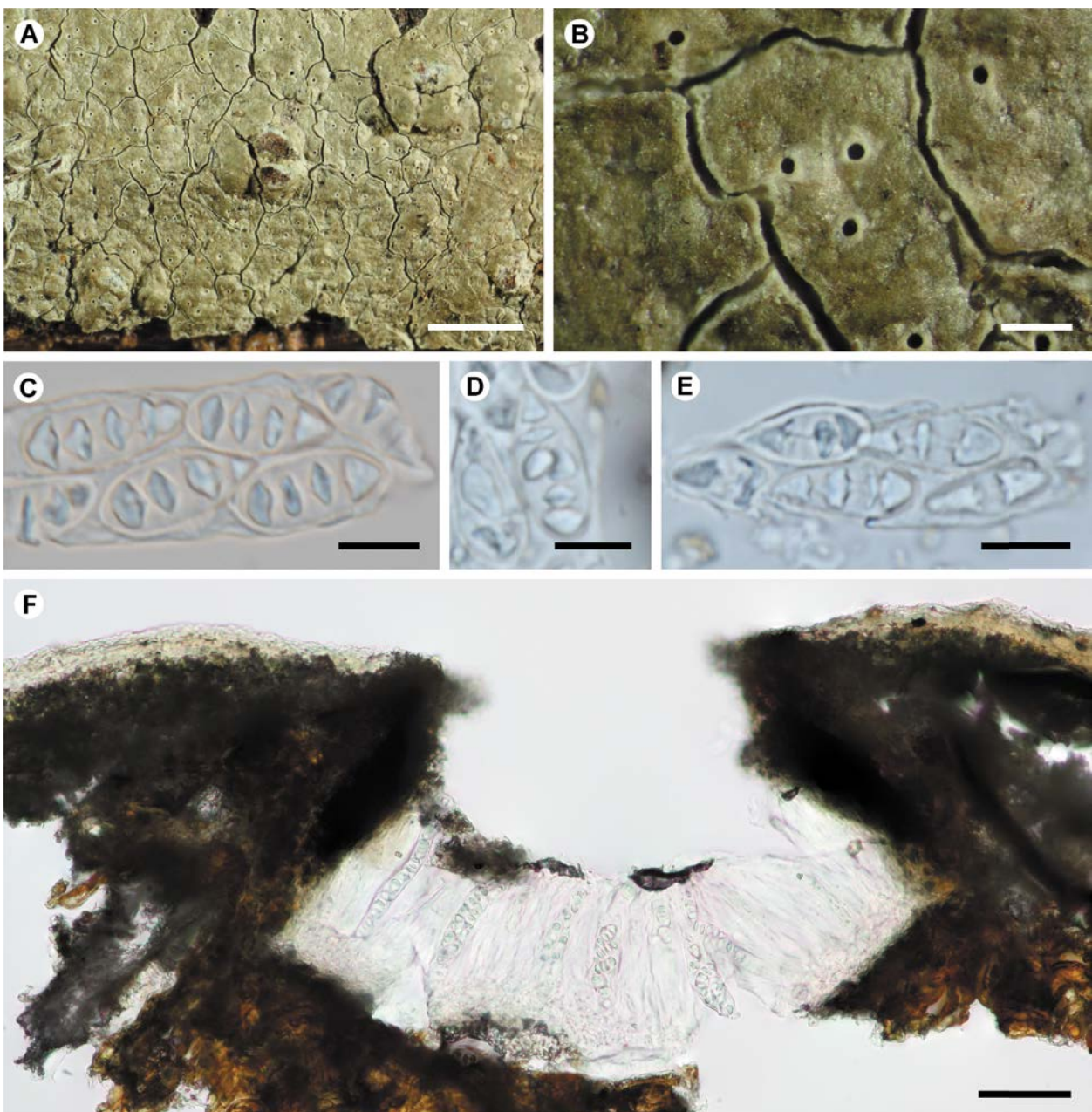


Figure 1. *Cladestinotrema carbonera* (holotype). A – thallus; B – pores concealing apothecia; C – mature ascospores with diamond-shaped lumina (tryptethelioid ascospores); D – mature ascospore showing a single longitudinal septum; E – immature ascospores showing *Caloplaca*-like development (discussed in Sipman et al. 2012); F – cross section of apothecium and thallus showing carbonized excipulum and dense cortex. Scales: A = 2 mm; B = 0.2 mm; C–E = 10 μm ; F = 50 μm .

Yellow efflux when KOH added to slide of apothecial sections. UV–.

Etymology. The specific epithet refers to the locality of the type specimen, El Bosque San Eusebio-La Carbonera, used here as a noun in apposition. A portion of this forest is maintained as a research site by the Universidad de Los Andes (Venezuela), Instituto de Silvicultura de la Facultad de Ciencias Forestales.

Ecology and distribution. This species is currently known only from the type specimen, which was collected on bark in montane cloud forest near Mérida, Venezuela, at approximately 2300–2400 m a.s.l. (see Barthlott et al. 2001 and León-Vargas et al. 2006 for descriptions of this area). This habitat is characteristic of *Clandestinotrema* species, which typically inhabit montane and cloud forest at higher elevations in the tropics (Frisch 2006; Mercado-Díaz et al. 2015). Cloud forest is an important lichen habitat in the Neotropics (Córdova-Cháves et al. 2014, 2016; Mercado-Díaz et al. 2015).

Remarks. The olive-green thallus distinguishes *Clandestinotrema carbonera* from other species in the genus, which typically have a white-gray to yellow-gray thallus. The new species is also unique in *Clandestinotrema* in having hypostictic acid as a major substance (Sipman et al. 2012). Hypostictic acid is present as an accessory substance in *C. antoninii*, which also has a dense cortex, lacks a columella, and has an excipulum carbonized in the upper half. However, that species has larger spores than *C. carbonera* ($25\text{--}45 \times 10\text{--}25 \mu\text{m}$ versus $15\text{--}24 \times 5.5\text{--}8.0 \mu\text{m}$) and also produces constictic and menegazziac acids. *Clandestinotrema erumpens* also lacks a columella and has accessory hypostictic acid, but the cortex is irregular, the excipulum is completely carbonized, and the spores are larger ($35\text{--}50 \times 15\text{--}20 \mu\text{m}$) and muriform. Finally, accessory hypostictic acid is present in *C. clandestinum* and *C. stylothecium*; like *C. carbonera*, these species occasionally produce submuriform ascospores, but they differ in having a columella (Sipman et al. 2012). Three additional species, *C. hepaticola*, *C. pauperius* and *C. tenue*, occasionally produce submuriform ascospores; the first of these differs from the new species in its hepaticolous substrate preference and lack of secondary chemistry, while the latter two differ in having a columella (Lücking 2015; Sipman et al. 2012).

Mason Hale apparently recognized this species as a new '*Thelotrema* sp.' (Keogh 1978) but never gave it a formal description; it is one of the two lichen species – along with *Xanthoparmelia quintaria* – in which naturally occurring hypostictic acid was first identified (Keogh 1978). Therefore there may be additional specimens (possible isotypes?) of this species at US, although a search for them was unsuccessful (several specimens of '*Thelotrema* sp.' collected in Mérida by Keogh appear in the US online database at <https://collections.nmnh.si.edu/search/botany/>, but the physical specimens cannot be located). Another specimen (possible isotype?) of this species, not examined for this study, may be at MER under number Keogh 20001.

Clandestinotrema pauperius (Nyl.) Rivas Plata, Lücking & Lumbsch in Rivas Plata et al., *Fung. Diversity* 52: 118. 2011.

Basionym: *Thelotrema pauperius* Nyl., *Ann. Sci. Nat. Bot.*, Sér. 5, 7: 318. 1867.

Type: Colombia, Lindig s.n. (H-NYL 22607, not seen; isotype: M, not seen).

Remarks. In addition to the new species described above, two additional *Clandestinotrema* species have been reported from El Bosque San Eusebio–La Carbonera: *Thelotrema 'neoleucomelanum'* and *C. leucomelaenum* (López Figueiras 1986; Maracano et al. 1996). The former is a nomen nudum, and the specimen cited by López Figueiras (1986) in reference to this name (Hale 42091) cannot be located. The latter report is based on a specimen at US which upon examination was found to represent *C. pauperius*. This species differs from *C. leucomelaenum* in having smaller ascospores with only a single longitudinal septum (Sipman et al. 2012).

Specimen examined. VENEZUELA. Mérida. Virgin bosque humedo forest of Universidad de los Andes, La Carbonera, 2500 m, 22 Jan. 1974, M. E. Hale 42080 (US).

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