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Guayaquilia gen. nov., typified by *Idriella cubensis*

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Abstract—A new genus *Guayaquilia* is established to accommodate *Idriella cubensis* based on morphology and phylogenetic analysis. DNA sequence data place these specimens as incertae sedis separate from *Microdochiaceae* (*Xylariales*), forming a monophyletic lineage separated from *Neoidriella desertorum* and phylogenetically distant from *Idriella*. The novel genus is characterized by macronematous, tree-like, fasciculate, profuse dichotomously, alternately, or irregularly branched, brown conidiophores with polyblastic, denticulate, sympodial extended, intercalary and terminal conidiogenous cells that produce solitary, sublunate, subnavicular, lunate, inequilateral, (0–)1-septate, hyaline conidia.

Key words—asexual fungi, hyphomycetes, *Microdochiaceae*, taxonomy, tropics

Introduction

Idriella, introduced by Nelson & Wilhelm (1956) with *I. lunata* as type species, is distinguished by macronematous, mononematous, unbranched, and basally

inflated and apically slightly geniculate brown conidiophores, mostly reduced to polyblastic, denticulate, sympodial extended conidiogenous cells. Conidia are typically lunate or falcate, unicellular, hyaline, and acuminate towards the apex (Nelson & Wilhelm 1956). Morgan-Jones (1979) updated and provided brief nomenclatural commentaries on *Idriella australiensis*, *I. couratarii*, *I. desertorum*, *I. lunata, I. mycogonoidea, I. ramosa, I. vandalurensis,* and *I. variabilis* and proposed two new species, *I. angustispora* and *I. bambusae*. Von Arx (1981), who presented a combined key for *Idriella* and *Microdochium* species, proposed the conidial shape and the habitat as the main characters to separate the genera; he included the *Idriella* species treated by Morgan-Jones (1979), excluded *I. tropicalis* as similar to *I. variabilis*, and added five new combinations (*I. bolleyi*, *I. caespitosa, I. falcata, I. grisea*, and *I. tainanensis*). Subsequently, 16 new species have been described *I. acerosa, I. amazonica, I. angamosensis, I. asaicola, I. cagnizarii, I. campnospermae, I. cubensis, I. euterpes, I. jambosae, I. licualae, I. mycophila, I. rara, I. rhododendri, I. setiformis, I. stilboidea,* and *I. uncinospora* (Castañeda-Ruiz 1985, 1986; Castañeda-Ruiz & Arnold 1985; Castañeda-Ruiz & Kendrick 1991; Castañeda-Ruiz & al. 1997; Matsushima 1985, 1995; Rodrigues & Samuels 1990, 1992; Subramanian & Bhat 1987; Wu & al. 1997).

When the generic concept of *Idriella* was expanded, it became a heterogeneous genus (Seifert & al 2011). In the generic type, *I. lunata*, conidiophores are unbranched and mostly reduced to conidiogenous cells producing unicellular conidia on tiny denticles arranged along the apical and subapical surface of each conidiogenous cell after several sympodial holoblastic extensions (Nelson & Wilhelm 1956), but many other *Idriella* species produce conidiophores branched in various patterns, sympodial extensions of the conidiogenous cells confined to a small, nodose area at the apex, and conidiogenous loci as inconspicuous denticles that produce unicellular or septate conidia. DNA sequences of several *Idriella* species revealed the polyphyletic nature of this genus, showing phylogenetic separation between *I. lunata* (type species) and four other *Idriella* species, which consequently were transferred to four new genera: *Castanediella* Hern.-Restr. & al., *Idriellopsis* Hern.-Restr. & Crous, *Neoidriella* Hern.-Restr. & Crous, and *Paraidriella* Hern.-Restr. & Crous (Crous & al. 2015, Hernández-Restrepo & al. 2016).

During a workshop on fungal diversity conservation in Cacao forest areas, two surveys of microfungi were conducted in *Theobroma cacao* plantations in Guayaquil, Guayas Province, Ecuador, and several *Idriella*-like specimens were found. LSU and ITS sequence analyses place these specimens as incertae sedis

and separate from *Microdochiaceae* (*Xylariales*). They are not congeneric with *Idriella lunata* (the generic type), and we propose a new genus *Guayaquilia* to accommodate them.

Materials & methods

Sampling and fungal strains studied

Samples of decaying plants were placed in plastic bags, transported to the laboratory, placed in moist chambers, and treated according to Castañeda-Ruiz & al. (2016). Individual conidia were separated from the plant material under a stereoscope using an entomological needle and cultured in two different media: V8 (V8: 125 ml V8 juice, 18 g agar, 1000 ml distilled water, pH 6.3) and Cornmeal agar (CMA: 20 g cornmeal, 18 g agar, 1000 ml distilled water, pH 6.3). Morphological observations were made from cultures grown on CMA after five days, incubated at 25 ± 1°C under 12 h alternating near-UV light and darkness (using Vica, FLB-20W T10 near-UV lamp in an irradiation box). Colony colors were coded according to Rayner (1970). Mounts were prepared in lactic acid (90%) or in polyvinyl alcohol-glycerol (8 g PVA in 100 mL of water + 5 mL of glycerol) and lactofuchsin (0.1 g acid fuchsin, 100 mL 85% lactic acid) (Carmichael 1955). Microscopic characters were measured at ×1000 using a Nikon Eclipse Ni-U microscope with DIC optics and a Nikon DS-Fi2 camera.

Pure cultures were deposited at the Microbial Culture Collection of the Biotechnology Research Center, Guayaquil, Ecuador (CCM-CIBE), and the voucher specimens and slide preparations were deposited in the Herbarium, Departamento de Micología, Universidade Federal de Pernambuco, Recife, Brazil (URM). Also examined were the holotype and another specimen deposited as *Idriella cubensis* in the INIFAT Fungus Collection.

DNA extraction, sequencing, and phylogenetic analysis

Genomic DNA was extracted from fresh mycelia grown on V8 Agar at 25°C using a modified rapid extraction method for filamentous fungi according to Cenis (1992). The primer pairs ITS1/ITS4 (White & al. 1990) and LROR/LR5 (Vilgalys & Hester 1990) were used for the amplification of the internal transcribed spacers (ITS), and part of the large subunit of the nuclear ribosomal RNA gene (LSU). PCR cycling conditions followed Korabecna (2007; ITS) and Yang & al. (2017; LSU). PCR products were sent to Macrogen Korea for purification and sequencing with the same primers.

Sequences were aligned and edited using MEGA v.6.0. Phylogenetic relationships among taxa were addressed through Maximum Likelihood (ML) analysis using MEGA v.6.0 and Bayesian analysis using Beast v1.10.4 (Drummond & al. 2012). Data for each gene or region was analyzed both individually and together as a combined data set. jModeltest v.2.1.10 (Darriba & al. 2012) was used to determine the best nucleotide substitution model for both studies. Congruence between individual gene data sets was tested using the partition homogeneity test (Farris & al. 1995) implemented in PAUP v4.0b10 (Swofford, 2001), using a heuristic search option with random taxon addition and TBR branch swapping with 1000 replicates. Tree topologies from ML

analysis of individual genes were also compared visually for congruence. For the combined ML study, GTR was specified as the evolutionary model with estimated proportion of invariables sites and gamma distribution as default parameters. Number of substitution rates was set to four and topology search changed to SPR for tree improvement. Nodal support was assessed by bootstrap analysis from 1000 replicates. Bootstrap values equal or higher than 70% were considered significant. Bayesian inference was run with four Monte Carlo Markov (MCM) chains over 1 million generations with a sampling frequency of 1000 trees using the GTR+I+G as the best model (Drummond & al. 2012). To detect if the sample distribution has reached stationarity, convergence and the effective sample size (ESS) were checked for each run. Posterior probabilities (PP) for the Bayesian analysis were determined by calculating 50% majority rule consensus tree and added onto congruent nodes of the ML tree topology. PP equal or above 0.95 were considered significant. Relevant sequences of ITS and LSU from Hernández-Restrepo & al. (2016) obtained from GenBank, were included in the phylogenetic inference for comparison purposes (Table 1).

Table I. Sequences of *Guayaquilia cubensis* and related species used in the phylogenetic analysis. New sequences are indicated in bold.

Species	Strain #	ITS	LSU	Reference
Astrocystis concavispora	IT1612	KP297404	KP340545	Daranagama & al. 2015
Castanediella acaciae	CBS 139896	KR476728	KR476763	Crous & al. 2015
C. cagnizarii	CBS 542.96	KP859054	KP858991	Hernández-Restrepo & al. 2016
	CBS 101043	KP859051	KP858988	Hernández-Restrepo & al. 2016
C. couratarii	CBS 579.71	KP859050	KP858987	Hernández-Restrepo & al. 2016
Guayaquilia cubensis	MUCL 39017	KC775733	KC775708	Becerra-Hernández & al. 2016
	CCMCIBE-H312	MH777025	MH777024	This Study
	CCMCIBE-H320	MH777026	MH777023	This Study
Idriella lunata	CBS 177.57	KP859043	KP858980	Hernández-Restrepo & al. 2016
	CBS 204.56	KP859044	KP858981	Hernández-Restrepo & al. 2016
	CBS 209.60	KP859045	KP858982	Hernández-Restrepo & al. 2016
	CBS 736.74	KP859046	KP858983	Hernández-Restrepo & al. 2016
Idriellopsis uncinospora	CBS 575.92	KP859052	KP858989	Hernández-Restrepo & al. 2016
Kretzschmaria deusta	CBS 163.93	KC477237	KY610458	Wendt & al. 2018

Microdochium albescens	CBS 243.83	KP858994	KP858930	Hernández-Restrepo & al. 2016
M. citrinidiscum	CBS 109067	KP859003	KP858939	Hernández-Restrepo & al. 2016
M. lycopodinum	CBS 109398	KP859005	KP858941	Hernández-Restrepo & al. 2016
M. majus	CBS 741.79	KP859001	KP858937	Hernández-Restrepo & al. 2016
M. neoqueenslandicum	CBS 445.95	KP858997	KP858933	Hernández-Restrepo & al. 2016
M. nivale	CBS 116205	KP859008	KP858944	Hernández-Restrepo & al. 2016
M. seminicola	CBS 122707	KP859007	KP858943	Hernández-Restrepo & al. 2016
Microdochium sorghi	CBS 691.96	KP859000	KP858936	Hernández-Restrepo & al. 2016
M. tainanense	CBS 269.76	KP859009	KP858945	Hernández-Restrepo & al. 2016
M. trichocladiopsis	CBS 623 77	KP858998	KP858934	Hernández-Restrepo & al. 2016
Neoidriella desertorum	CBS 985.72	KP859048	KP858985	Hernández-Restrepo & al. 2016
Paraidriella jambosae	CBS 374.90	KP859049	KP858986	Hernández-Restrepo & al. 2016
Poronia punctata	CBS:656.78	KT281904	KY610496	Wendt & al. 2018
Rosellinia aquila	MUCL 51703	KY610392	KY610460	Wendt & al. 2018
R. corticium	MUCL 51693	KY610393	KY610461	Wendt & al. 2018
R. necatrix	CBS 349.36	AY909001	KF719204	Peláez & al. 2008
Sarcoxylon compunctum	CBS 359.61	KT281903	KY610462	Wendt & al. 2018
Selenodriella cubensis	CBS 683.96	KP859053	KP858990	Hernández-Restrepo & al. 2016
S. fertilis	CBS 772.83	KP859055	KP858992	Hernández-Restrepo & al. 2016
Xylaria arbuscula	CBS:126415	KY610394	KY610463	Fournier & al. 2011; Wendt & al. 2018
X. hypoxylon	CBS 122620	KY610407	KY610495	Wendt & al. 2018
X.polymorpha	MUCL 49884	KY610408	KY610464	Wendt & al. 2018

Phylogeny

The LSU-ITS dataset comprised 36 aligned sequences with 1482 positions. Fig. 1 presents the ML tree including BS and PP values. The partition homogeneity test showed no significant incongruence between the combined data sets ($p > 0.05$). Trees obtained from ML and Bayesian analysis of the individual loci and the combined analysis produced congruent topologies.

Phylogenetic inferences grouped the three strains identified as *Idriella cubensis* (MUCL 39017, CCMCIBE-H312, CCMCIBE-H320) together in a

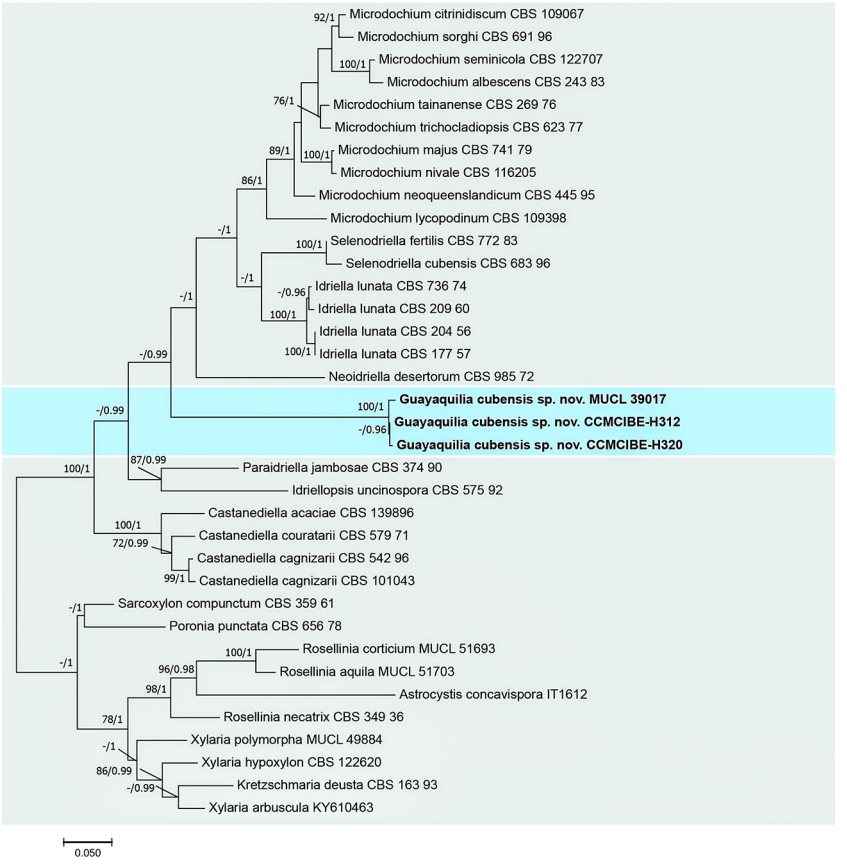

Fig. 1. Maximum Likelihood (ML) non-rooted tree inferred from the combined LSU and ITS sequences of *Guayaquilia cubensis* and related species. Numbers on the branches are support values >75% from BS and >0.95 from PP. The scale bar shows the expected changes per site.

supported clade and sister to *Neoidriella desertorum*, both clades separate from, and basal to, the *Microdochiaceae* Hern.-Restr. & al. clade containing *Idriella lunata*, *Microdochium* spp., and *Selenodriella* spp. We therefore propose a new genus *Guayaquilia* typified by *Idriella cubensis*.

Taxonomy

Guayaquilia R.F. Castañeda, Magdama, D. Sosa & Hern.-Restr., **gen. nov.**

MB 831849

Differs from *Neodriella* and *Paraidriella* by its macronematous, tree-like, irregularly multibranched conidiophores and its intercalary, discrete conidiogenous cells.

Type Species: *Idriella cubensis* R.F. Castañeda & G.R.W. Arnold [≡ *Guayaquilia cubensis* (R.F. Castañeda & G.R.W. Arnold) R.F. Castañeda & al.].

Etymology: *Guayaquilia* (Latin), referring to Guayaquil city, Ecuador.

Asexual. Colonies on natural substrate effuse, pulvinate-velutinous, golden brown to brown. Conidiophores macronematous, mononematous, fasciculate, erect, cylindrical, irregularly branched, septate, smooth, brown. Conidiogenous cells polyblastic, denticulate, sympodial extended, terminal or intercalary, cylindrical or slightly inflated, pale brown to brown. Conidial secession schizolytic. Conidia solitary, acropleurogenous, sublunate, subnavicular, inequilateral, unicellular or septate, hyaline, smooth. Chlamydospores solitary, bicellular, brown and the apical cell globose, thick walled, smooth.

Guayaquilia cubensis (R.F. Castañeda & G.R.W. Arnold) R.F. Castañeda, Magdama, D. Sosa & Hern.-Restr., **comb. nov.** Figs 2, 3

MB 831851

≡ *Idriella cubensis* R. F. Castañeda & G.R.W. Arnold, Rev. Jard. Bot. Nac., Univ. Habana 6(1): 50 (1985).

Colonies on V8 agar at 25°C attaining 60 mm diam after five days, floccose, pale lavender grey to buff, sporulation poor, sparse. On CMA reaching 50 mm diam, felted, dark brick at the center, whitish toward the periphery. Conidiophores macronematous, mononematous, tree-like, fasciculate, profuse, dichotomously, alternately or irregularly branched toward the apex, slightly inflated or bulbous at the base, smooth, brown below, pale brown to subhyaline at the tip of branches, <500 µm long, 4–7.5 µm wide at the base, branches 2–3.5 µm wide. Conidiogenous cells polyblastic, denticulate, intercalary and terminal, in branches integrated, cylindrical, or slightly curved, brown to pale brown or subhyaline, 8–25 × 2–3 µm. Conidia solitary, acropleurogenous, sublunate, subnavicular, lunate, inequilateral, (0–) 1-septate, 14–17 × 2.5–3 µm. Chlamydospores bicellular, the basal cells subcampanulate, subcuneiform to hemispherical, 5–8 µm wide, and the apical cells globose, 18–22 µm diam, brown, thick walled; both cells with lumina granulose, brown; terminal, smooth, arising from assimilative hyphae.

specimens examined: **ECUADOR, Guayas Province, Naranjal**, 2°48′S 79°40′W, on decaying leaves of *Theobroma cacao* L. (*Malvaceae*), 8 July 2017, F. Espinoza & S. Pérez-Martínez (URM 91815 = CCMCIBE-H312; GenBank MH777025, MH777024); 2°41′S 79°36′W, on decaying leaves of *Theobroma cacao*, 8 July 2017, F. Espinoza & S. Pérez-Martínez (URM 91815a = CCMCIBE-H320; GenBank MH777026, MH777023). **CUBA, La Habana Province, Santiago de Las Vegas**, 22°58′N 82°22′W, on decaying leaves of *Calophyllum calaba* L. (*Clusiaceae*), 29 Jun 1983, RF Castañeda Ruiz (holotype,

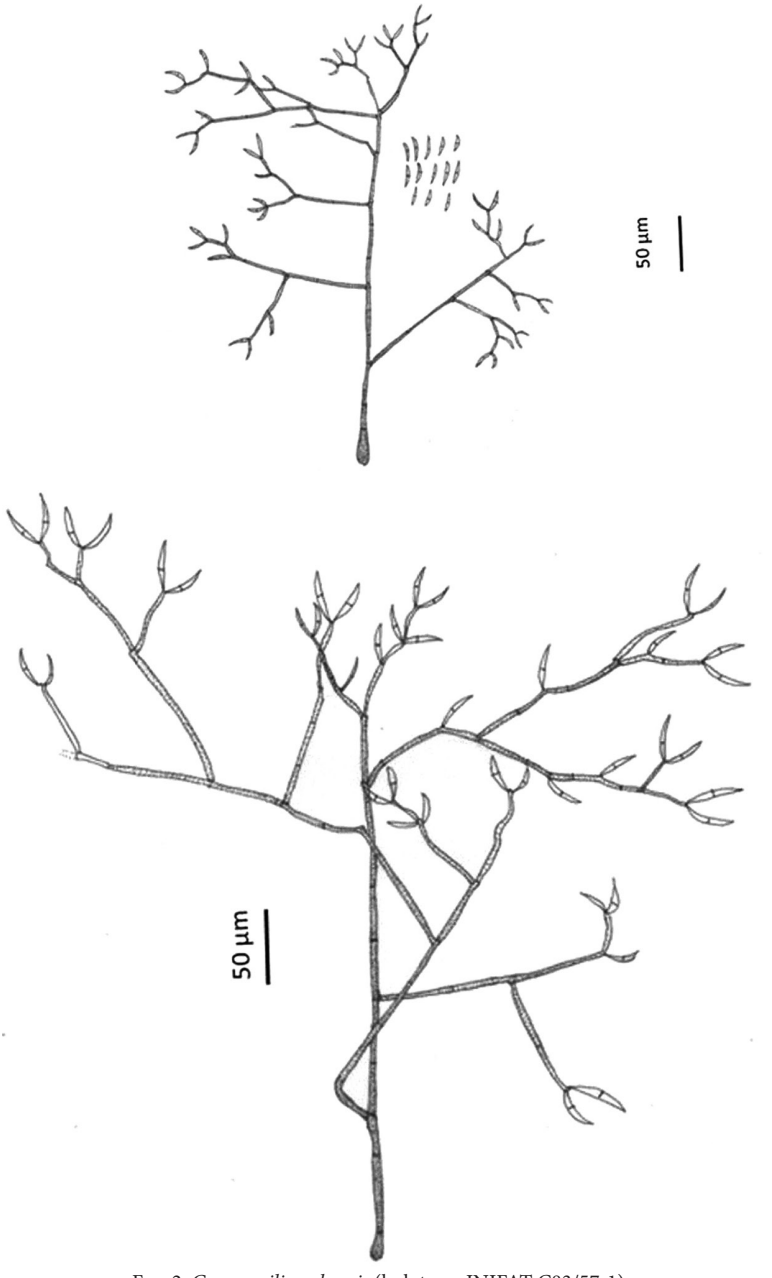

Fig. 2. *Guayaquilia cubensis* (holotype, INIFAT C83/57-1). Conidiophores, conidiogenous cells, and conidia.

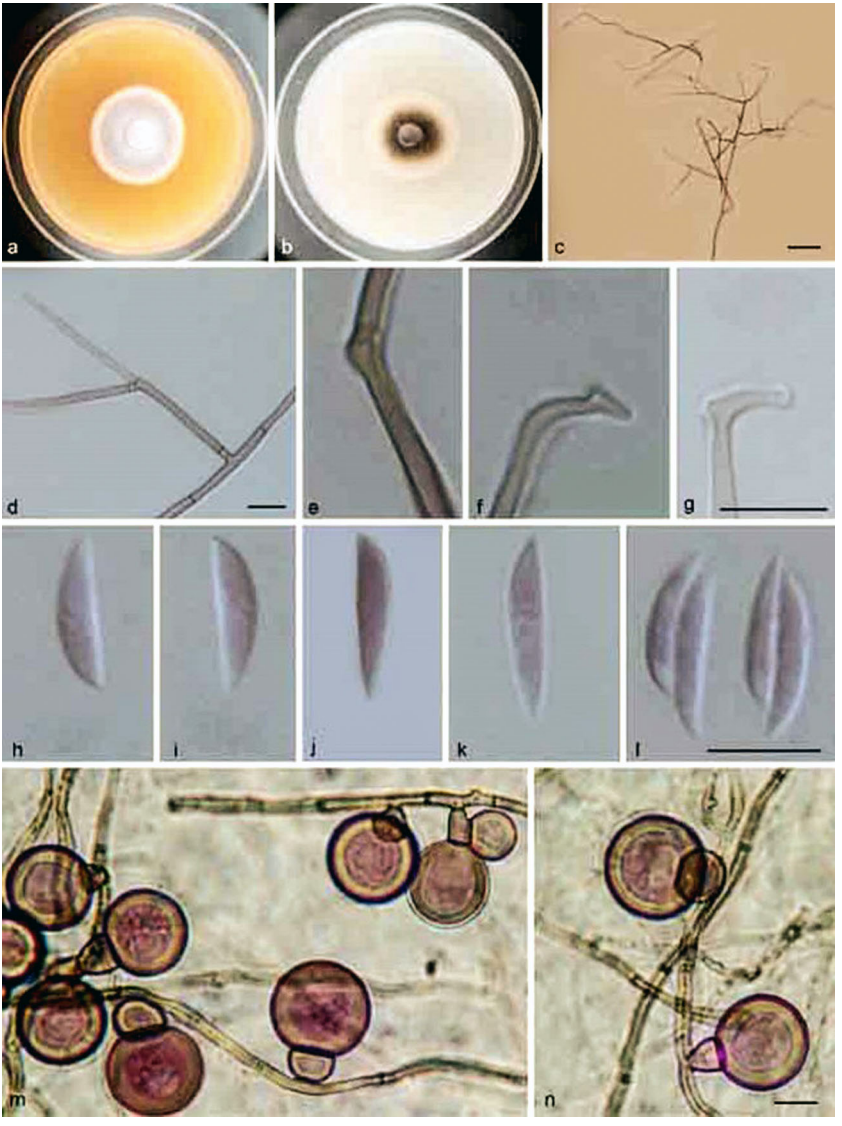

Fig. 3. *Guayaquilia cubensis* (CCMCIBE-H312). A. Colony on V8 agar; B. Colony on CMA; C, D. Conidiophores; E–G. Conidiogenous cells; H–L. Conidia; M, N, Chlamydospores. Scale bars = 10 μm.

INIFAT C83/57-1); **Pinar del Rio Province**, **Los Portales**, 22°40′N 83°28′W, on decaying leaves of *Cupania* sp. (*Sapindaceae*), 5 Feb. 1994, RF Castañeda Ruiz, (INIFAT C94/27 = MUCL 39017).

Note: *Guayaquilia* shares some morphological characters with the genera *Castanediella*, *Idriella*, *Idriellopsis*, *Neoidriella*, and *Paraidriella*, such as blastic conidial ontogeny and denticulate conidiogenous loci, but the tree-like, dichotomously, alternately or irregularly profuse branched conidiophores with intercalary and terminal conidiogenous cells are present only in *Guayaquilia*. Also, the bicellular, globose, brown chlamydospores that arise from assimilative hyphae in *Guayaquilia* are another distinctive character. The conidiophore morphology in *Guayaquilia* resembles that of some *Phaeodactylium* species (*P. biseptatum*, *P. curvularioides*, and *P. stadleri*) described by Castañeda-Ruiz & al. (2009, 2013) and Matsushima (1980), but these taxa have clavate, ellipsoidal, to obovoid, brown to subhyaline conidia.

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