Research article

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Two new species and a new record of the asexual micromycete genus *Endophragmiella* from Mexico

Dos nuevas especies y un registro nuevo del género de micromicetos asexuales *Endophragmiella* de México

Rosa María Arias[1], Rafael F. Castañeda-Ruiz[2,3], Gabriela Heredia[1,4]

Abstract:

Background and Aims: *Endophragmiella* species are saprobic asexual microfungi with a wide geographical distribution. Morphologically, the genus is characterized by simple or branched conidiophores, monoblastic, percurrently extending conidiogenous cells, and 0- or multiseptated conidia, variable in shape and seceding rhexolytically. The aim of this study was to describe two new species, *Endophragmiella chiapanensis* and *E. multiseptata*, and to record *E. gardeniae* for the first time from Mexico.

Methods: Plant debris was collected from the soil in areas of oak-pine forest in Chiapas and in a cloud forest in the state of Veracruz, Mexico. Material was transported to the laboratory and incubated in moist chambers. Microscopic slides of the sporophores were made, and spores were simultaneously transferred to plates with culture media. Reference materials (microscopic preparations) were deposited in the herbarium XAL of the Instituto de Ecología, A.C. in Xalapa, Veracruz, Mexico.

Key results: Taxonomic determination was based on morphological analysis of sporophores. *Endophragmiella chiapanensis* is characterized by fusiform, mostly 3-euseptate, versicolorous conidia, brown to pale brown, often bearing a selenosporella-like synanamorph in the apical cell, whereas *E. multiseptata* is distinguished by cylindrical to obclavate, mostly 9-septate, concolorous, pale brown conidia. With the present contribution, 13 species of *Endophragmiella* have been reported from Mexico.

Conclusions: The species described as new taxa present conidiogenesis, secession and conidial morphological features typical of the genus *Endophragmiella*. However, conidial size and shape distinguish them from the remaining species described so far within the genus. *Endophragmiella* species have been poorly studied in Mexico. More field work and molecular studies are necessary to increase our knowledge of their diversity as well as to define their phylogenetic affinities.

Key words: anamorphic fungi, Ascomycota, cloud forest, conidial fungi, saprobes.

Resumen:

Antecedentes y Objetivos: Las especies de *Endophragmiella* son micromicetos asexuales saprobios con una amplia distribución geográfica. Morfológicamente el género se caracteriza por conidióforos simples o ramificados, células conidiógenas monoblásticas, percurrentes y conidios 0- o multiseptados, de forma variable y con secesión rhexolítica. El objetivo del estudio fue describir dos especies nuevas, *Endophragmiella chiapanensis* y *E. multiseptata*, y registrar *E. gardeniae* por primera vez para la micobiota de México.

Métodos: Se recolectaron del suelo restos vegetales en áreas de bosque de encino-pino en Chiapas y en un bosque de niebla del estado de Veracruz, México. El material se transportó al laboratorio y se incubó en cámaras húmedas. Se hicieron preparaciones microscópicas de los esporóforos y simultáneamente se transfirieron esporas a placas con medios de cultivo. El material de referencia (preparaciones microscópicas) se depositó en el herbario XAL del Instituto de Ecología, A.C. en Xalapa, Veracruz, México.

Resultados clave: La determinación taxonómica se basó en el análisis morfológico de los esporóforos. *Endophragmiella chiapanensis* se caracteriza por presentar conidios fusiformes, principalmente con 3-euseptos, versicoloridos, pardo a pardo claro, frecuentemente con un sinanamorfo tipo selenosporella en la célula apical, mientras que *E. multiseptata* se distingue por tener conidios cilíndricos y obclaviformes, mayormente 9-euseptos, concoloridos, color pardo claro. Con la presente contribución, 13 especies de *Endophragmiella* han sido registradas para México.

Conclusiones: Las especies descritas como nuevos taxones presentan conidiogénesis, secesión y morfología conidial típicas del género *Endophragmiella*. Sin embargo, el tamaño y forma de sus conidios las distinguen del resto de las especies descritas en el género hasta ahora. Las especies de *Endophragmiella* han sido pobremente estudiadas en México. Son necesarias más exploraciones en campo y estudios moleculares para incrementar nuestro conocimiento sobre su diversidad, así como para definir sus afinidades filogenéticas.

Palabras clave: Ascomycota, bosque de niebla, hongos anamorfos, hongos conidiales, saprobios.

[1]Instituto de Ecología, A.C., Red de Biodiversidad y Sistemática, Carretera antigua a Coatepec No. 351, Col. Congregación El Haya, 91073 Xalapa, Veracruz, México.
[2]Instituto de Investigaciones de Sanidad Vegetal, Calle 110 No. 514 e/5ta B y 5ta F, Playa, 11600 La Habana, Cuba.
[3]Academia de Ciencias de Cuba, Cuba No. 460 e/Amargura y Teniente Rey, Habana Vieja, 10100 La Habana, Cuba.
[4]Author for correspondence: gabriela.heredia@inecol.mx

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Introduction

Sutton (1973) established the genus *Endophragmiella* B. Sutton with *E. pallescens* B. Sutton as the type species. Later, Hughes (1979) emended the genus and detailed the conidiogenesis, conidium secession and percurrent proliferation of the conidiogenous cells in *E. pallescens* and *E. boewei* J.L. Crane ex S. Hughes. The genus is characterized by macronematous, mononematous conidiophores, solitary or in small groups, simple to irregularly branched, erect or slightly flexuous, septate, pale brown to dark brown, with percurrent extensions at the apex producing several annellations. Its conidiogenous cells are integrated, terminal, cylindrical, smooth, pale brown to brown and percurrent. Conidial secession is rhexolytic, and conidia are acrogenous, solitary, pale brown to dark brown, 0- or multiseptate, truncate at the base, and bearing a frill or ridge around the scar, which is composed of a detached portion of the apex of the conidiogenous cell (Hughes, 1979).

As for the vast majority of conidial micromycetes, the species of *Endophragmiella* are considered asexual forms of Ascomycetes (Seifert et al., 2011), but as will be discussed later, except for one case, their position into more specific taxonomic levels has not been elucidated. Around 110 species and one variety have been described in the genus (Index Fungorum, 2024). However, two of them, *E. fasciata* (R.F. Castañeda) R.F. Castañeda and *E. quadrilocularis* Matsush., were relocated to *Repetophragma fasciatum* (R.F. Castañeda) R.F. Castañeda, Gusmão & Saikawa (Castañeda Ruiz et al., 2006) and *R. quadriloculare* (Matsush.) R.F. Castañeda, McKenzie & K.D. Hyde (Castañeda-Ruiz et al., 2011), respectively. Likewise, *E. fuliginosa* (B. Sutton) S. Hughes was relocated to *Rhexoacrodictys fuliginosa* (B. Sutton) W.A. Baker & Morgan-Jones (Baker et al., 2002), *E. rigidiuscula* R.F. Castañeda to *Distophragmia rigidiuscula* (R.F. Castañeda) R.F. Castañeda, S.M. Leão & Gusmão (Castañeda-Ruiz et al., 2015), *E. socia* (M. B. Ellis) S. Hughes to *Sporidesmium socium* M.B. Ellis (Ellis, 1958), and *E. valdiviana* (Speg.) S. Hughes to *Camposporium valdivianum* (Speg.) G. Delgado & Koukol (Koukol and Delgado, 2021), remaining 104 species and one variety within the genus.

Most *Endophragmiella* species are saprobes, occurring in template and tropical environments on rotten wood, dead branches, and decaying leaves of many different plant species, whereas some species have been isolated from soil and detected overgrowing other fungi (Sutton, 1973; Hughes, 1979; Dunn, 1982; Jiang et al., 2018).

The objective of the present contribution is to describe two species as new, *Endophragmiella chiapanensis* and *E. multiseptata*, and to record *E. gardeniae* for the Mexican mycobiota. Additionally, a key is presented for the identification of *Endophragmiella* spp. known in Mexico.

Materials and Methods

Study area

Specimens of the new species were collected in an oak-pine forest in Huitepec Los Alcanfores (16°44'28''N, 92°40'44''W), municipality San Cristóbal de las Casas, Chiapas, Mexico, at an elevation of 2330 m a.s.l. (Fig. 1). The climate in the area is temperate sub-humid (García, 2004). The maximum temperature ranges between 18 and 21 °C from May to October and the minimum ranges between 3 and 6 °C from November to April, while the average annual precipitation is around 1300 mm, with peaks of rainfall during the summer (Ramírez-Marcial et al., 1998). Material of the new record was collected in a relic of cloud forest in Mesa de la Yerba (19°33'40''N, 97°01'04''W), municipality Acajete, Veracruz, Mexico, at an elevation of 1933 m a.s.l. (Fig. 1). The climate in this area is temperate humid with rain in summer; maximum temperature ranges between 21 and 23 °C from May to June and the minimum ranges between 9 and 12 °C from December to January; the average annual precipitation is 1457 mm (INEGI, 2024).

Sampling and sample handling

Plant debris was placed in paper bags, taken to the laboratory, and treated according to Castañeda-Ruiz et al (2016). Moist chambers were periodically examined under a stereomicroscope (Zeiss SV11, Jena, Germany). Permanent and semi-permanent slides were prepared using polyvinyl alcohol-glycerol (8 g PVA in 100 ml water, plus 5 ml

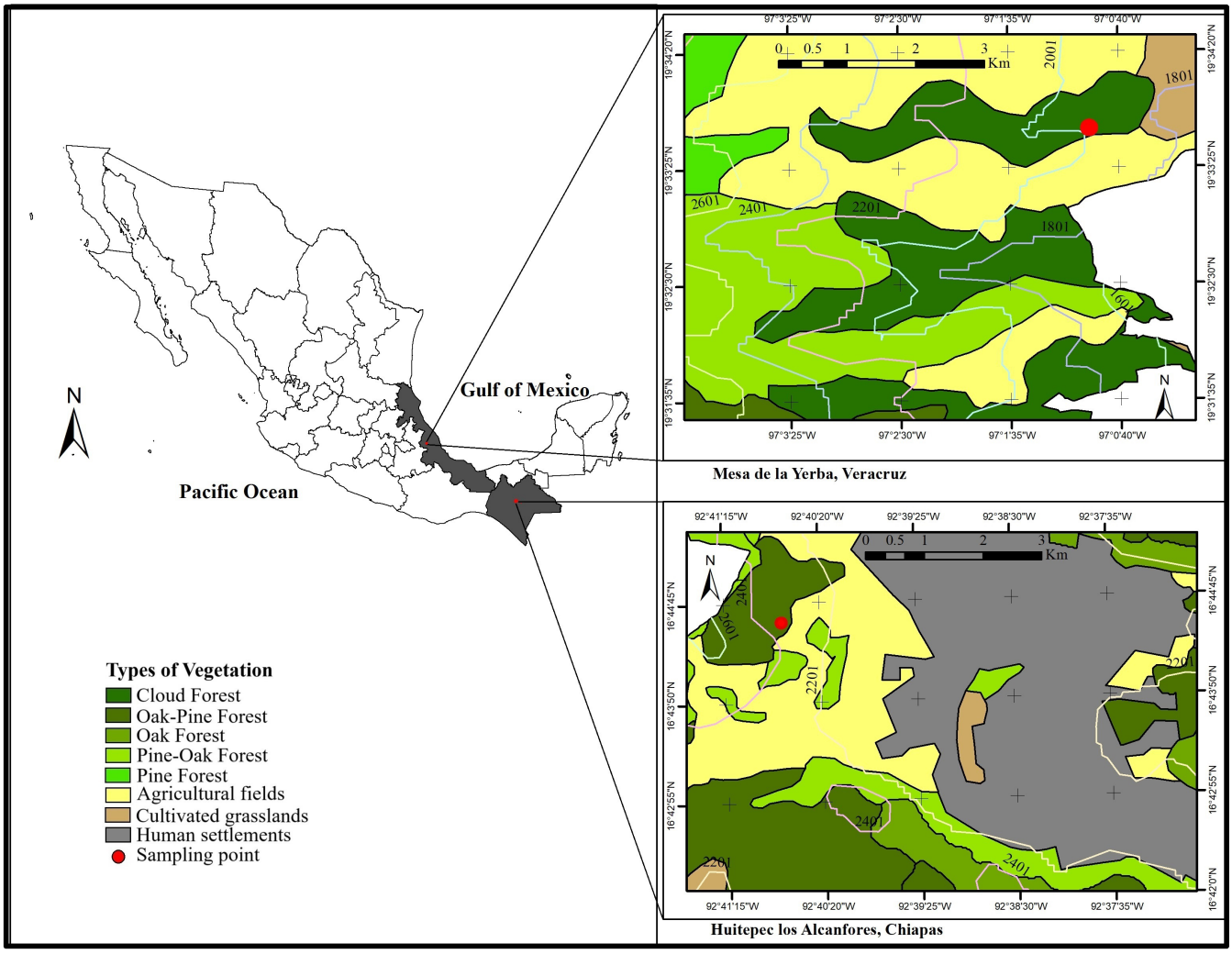

Figure 1: Collection areas, Huitepec, Los Alcanfores, San Cristóbal de las Casas, Chiapas and Mesa de la Yerba, Acajete, Veracruz, Mexico. Map created with ArcGIS ver. 10.4.1 (ArcGIS, 2015), using vector data on land use and vegetation from INEGI, VII series, Ed. 2018 (scale 1:250,000) (INEGI, 2024).

glycerol), lacto fuchsin (0.1 g acid fuchsin, 100 ml 85% lactic acid), and lactic acid (90%), following Carmichael (1955).

Measurements were taken under the 100× oil immersion lens using a Nikon Eclipse Ni-U microscope (Nikon, Tokyo, Japan) with an integrated software program NIS-Elements D (Nikon, Tokyo, Japan) that allows to obtain reliable measurements with decimals. Photomicrographs were taken with a Nikon DSFi2 digital camera (Nikon, Tokyo, Japan) installed on the Nikon Eclipse Ni-U microscope, and processed by using Photoshop v. 22.2 (Adobe Systems Incorporated, California, USA).

Holotypes were deposited in the herbarium XAL of the Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico. To obtain isolates, samples consisting of conidia were transferred to plates with potato dextrose agar (PDA; BD DifcoTM), corn meal agar (CMA; BD DifcoTM) and water agar, followed by incubation at room temperature. However, in all cases conidia remained dormant on the plates, so it was not possible to isolate the fungi. Species descriptions are based on their morphological characterization on natural substrate. A dichotomous taxonomic key was constructed based on the morphological characteristics of the conidia.

Results

Taxonomy

Ascomycota

Pezizomycotina

Sordariomycetes

Incertae sedis

Endophragmiella chiapanensis R.F. Castañeda, Heredia & R.M. Arias, sp. nov. Figs. 2, 3.

Index Fungorum 901465

TYPE: MEXICO. Chiapas, municipality San Cristóbal de las Casas, Huitepec Los Alcanfores, oak-pine forest, 2330 m, 16°44'28''N, 92°40'44''W, 21.X.2022, *G. Heredia CB2303* (holotype: XAL!).

Endophragmiella chiapanensis differs from *E. acuta* by its longer and narrower conidia (26.9-39.6 × 5.6-9 µm *vs.* 18-25 × 8-10 µm) and from *E. acutispora* (42.5-57.5 × 10-12.5 µm) by its shorter and narrower conidia.

Asexual morph, colonies on the natural substrate effuse, hairy, brown; mycelium partly immersed, composed of branched, septate, pale brown, smooth-walled hyphae, 1-2 µm wide; conidiophores macronematous, mononematous, solitary or in small groups, simple, erect, straight, or flexuous, up to 7-septate, smooth, brown, paler toward the apex, up to 175 µm high × 3.7-4.5 µm wide; conidiogenous cells monoblastic, integrated, terminal, cylindrical, smooth, pale brown, with 3-11 annellated, enteroblastic percurrent extensions; conidial secession rhexolytic; conidia solitary, acrogenous, fusiform or obclavate, rostrate, conico-truncate at base, 3-euseptate, versicolorous, central cells brown, basal cells brown to pale brown and apical cells hyaline, smooth, (26.9-)30-37(-39.6) µm long × (5.6-)6.2-8.5(-9) µm wide at the broadest part ($\bar{X}$=33.6 × 7.5 µm, n=60), with a distinct basal frill of 1-1.5 µm long; synanamorph selenosporella-like in the conidia apical cell, conidiogenous cell up to 4 µm long × 1-1.5 µm wide, conidia filiform, acerose, aseptate, hyaline (3.9-)5-6.6(-7) µm long × 0.5-0.9 µm wide ($\bar{X}$=5.9 × 0.77 µm, n=15).

Sexual morph: unknown.

Habit and habitat: saprobic, growing on dead herbaceous stems in an oak-pine forest.

Etymology: the species epithet refers to Chiapas state, where the species has been found.

Taxonomic notes: a comparison of *Endophragmiella* species with similar conidial morphology and production of selenosporella-like synanamorphs is summarized in Table 1. Conidial shape and the synanamorph position in *E. acutispora* Chuaseehar., Somrith. & Boonyuen (Chuaseeharonnachai et al., 2022) and *E. acuta* W.P. Wu (Wu and Zhuang, 2005) are similar to *E. chiapanensis.* However, conidial dimensions are quite different; conidia in *E. acutispora* are larger (42.5-57.5 × 10-12.5 µm), while in *E. acuta* they are shorter and slightly wider (18-25 × 8-10 µm) than those found in the new taxon. Furthermore, mature conidia in *E. acutispora* are 4-septate.

On the other hand, although the range of the conidial length of *E. canadensis* (Ellis & Everh.) B. Sutton (Sutton, 1973) and *E. cesatii* (Mont.) S. Hughes (Hughes, 1979) overlaps well with those of the new species, the width of their conidia is noticeably greater (12-15 µm and 9-13 µm, respectively) compared with those found in *E. chiapanensis*. Additionally, *E. canadensis* has verrucose conidia (Sutton, 1973), and the synanamorph in *E. cesatii* can be located singly or in groups apically and laterally on short separate stalks (≤30 µm long), producing falcate, unicellular (8-10 × 1 µm), hyaline conidia (Hughes, 1979). Likewise, *E. selenosporellaria* Y.R. Ma & X.G. Zhang, besides having branched conidiophores, is characterized by shorter and thinner conidia (14.5-22 × 4.5-6.5 µm) (Ma et al., 2015) than those in *E. chiapanensis*.

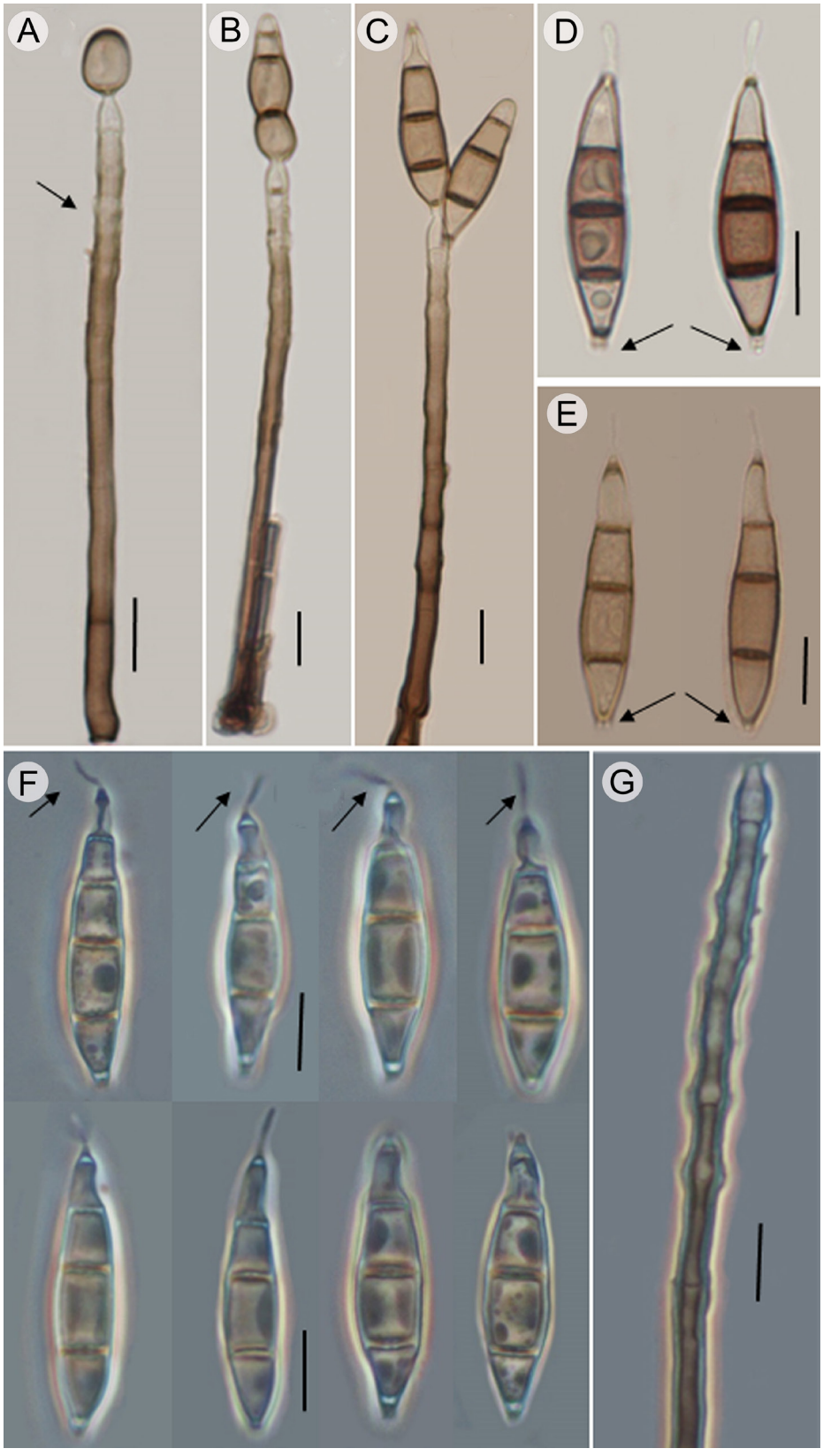

Figure 2: *Endophragmiella chiapanensis* R.F. Castañeda, Heredia & R.M. Arias. A. conidiophore with conidiogenous cells and immature conidia, showing successive percurrent extensions (arrows); B-C. conidiophore with conidia; D-E. conidia with basal frills (arrows); F. conidia in phase contrast with and without selenosporella-like synanamorph in the apical cells (arrows); G. conidiophore in phase contrast showing successive percurrent extensions. Scale bars=10 µm. Photos by G. Heredia of *G. Heredia CB2303* (XAL).

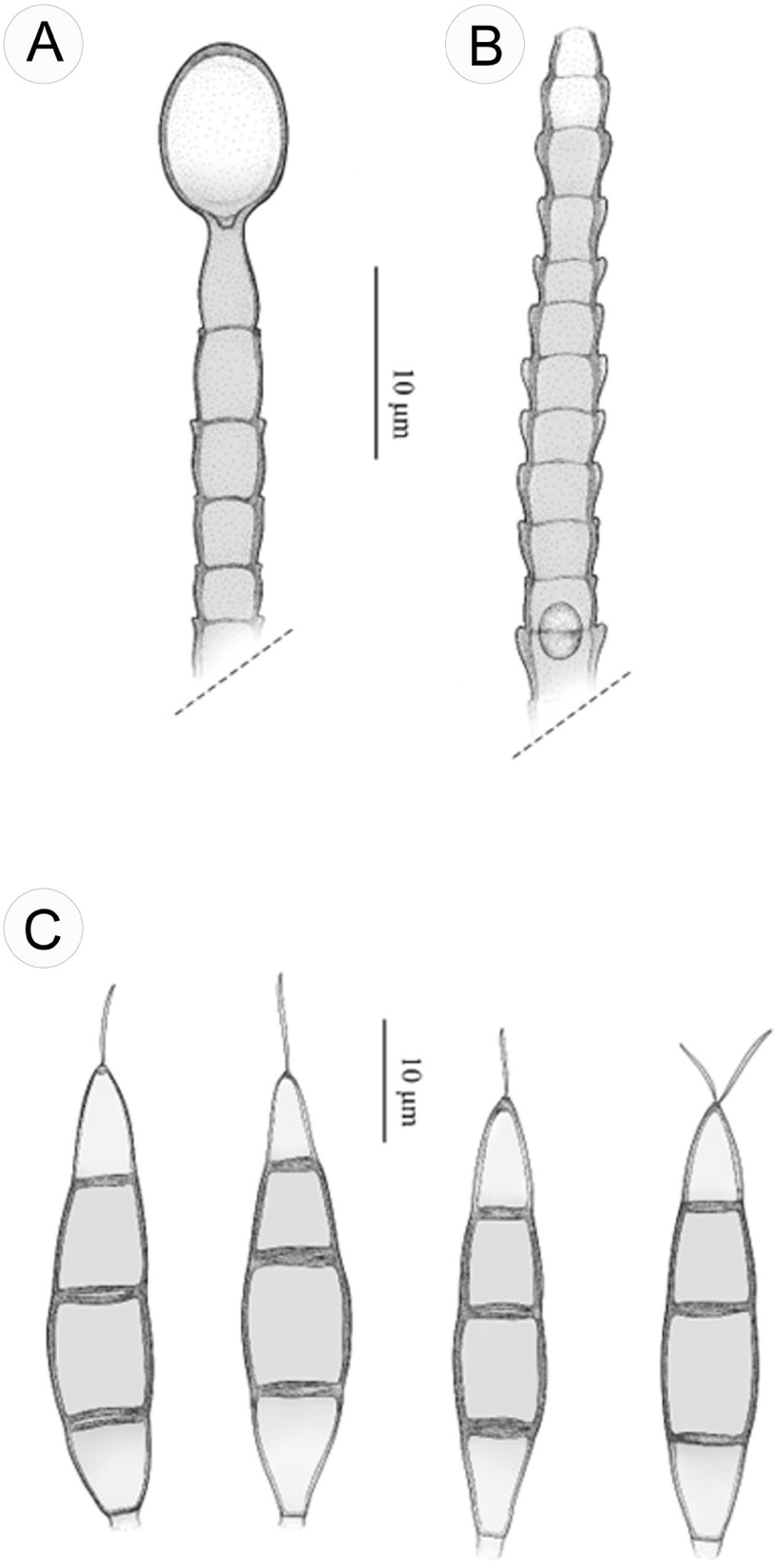

Figure 3: Line drawings of *Endophragmiella chiapanensis* R.F. Castañeda, Heredia & R.M. Arias. A. conidiophore bearing an immature conidium; B. conidiophore with multiple percurrent extensions; C. mature conidia with basal and apical cells paler than the central cells, apical cell bearing spores of a selenosporella-like synanamorph. Scale bars=10 µm. Drawn by María Teresa Jiménez Segura based on the holotype.

Table 1: Comparison of the conidial morphology of *Endophragmiella* spp. with features similar to *Endophragmiella chiapanensis* R.F. Castañeda, Heredia & R.M. Arias. The new species is highlighted in bold.

Taxon	Conidial characteristics					
	Shape	Septation	Pigmentation	Dimensions (µm)	Synanamorph	Reference
Endophragmiella acuta W.P. Wu	Obclavate or fusiform, rostrate apex acute	3 constricted at septa	Versicolorous, brown to dark brown, apical cell paler	18-25 × 8-10	Apical cell sometimes with selenosporella-like conidia	Wu & Zhuang, 2005
Endophragmiella acutispora Chuaseehar., Somrith. & Boonyuen	Fusiform, obclavate, apex acute and elongated	(2-)4(-5)	Versicolorous, central cells brown, basal and apical cells paler	(42.5-)47.5-57.5 × 10-12.5	Apical cell often bearing selenosporella-like conidia 7.5-12.5×1.3 µm	Chuaseeharonnachai et al., 2022
Endophragmiella canadensis (Ellis & Everh.) B. Sutton	Fusiform to pyriform, verruculose	2-3	Versicolorous, pale brown with the central cells darker than the apical ones	23.5-35 × 12-15	Conidiophores and conidial apical cell bearing selenosporella-like conidia 10-20×0.9-1.3 µm	Sutton, 1973
Endophragmiella cesatii (Mont.) S. Hughes	Ellipsoid to fusiform	(2-)3(-4)	Versicolorous, central cells brown, basal and apical cells paler	22-44 × 9-13	Apical cell with selenosporella-like conidia apically and laterally on short stalks 8-10×1 µm	Hughes, 1979
***Endophragmiella chiapanensis* R.F. Castañeda, Heredia & R.M. Arias**	**Fusiform, obclavate, rostrate apex acute**	**3**	**Versicolorous, central cells brown, basal cell pale brown and apical cell hyaline**	**(26.9-)30-37 (-39.6) × (5.6-)6.2-8.5 (-9)**	**Apical cell often bearing selenosporella-like conidia (3.9-)5-6.6(-7)×0.5-0.9 µm**	**This study**
Endophragmiella selenosporellaria Y. R. Ma & X. G. Zhang	Elongated ellipsoid to obclavate	(0-)2-3	Versicolorous, basal cell brown to dark brown, apical cell subconical elongated, subhyaline to hyaline	14.5-22 × 4.5-6.5	Apical cell often with selenosporella-like conidia 8-12×1-1.5 µm	Ma et al., 2015

Endophragmiella multiseptata R.F. Castañeda, Heredia & R.M. Arias, sp. nov. Figs. 4, 5.

Index Fungorum 901466

TYPE: MEXICO. Chiapas, municipality San Cristóbal de las Casas, Huitepec Los Alcanfores, oak-pine forest, 2330 m, 16°44'28''N, 92°40'44''W, 21.X.2022, *G. Heredia CB2304* (holotype: XAL!).

Endophragmiella multiseptata differs from all known *Endophragmiella* species by its cylindrical to long obclavate, mostly 9-septate conidia.

Asexual morph, colonies on the natural substrate effuse, hairy, brown; mycelium partly immersed, composed of branched, septate, brown, smooth-walled hyphae, 2-3.5 µm wide; conidiophores macronematous, mononematous, solitary or in small groups, simple, erect, straight,

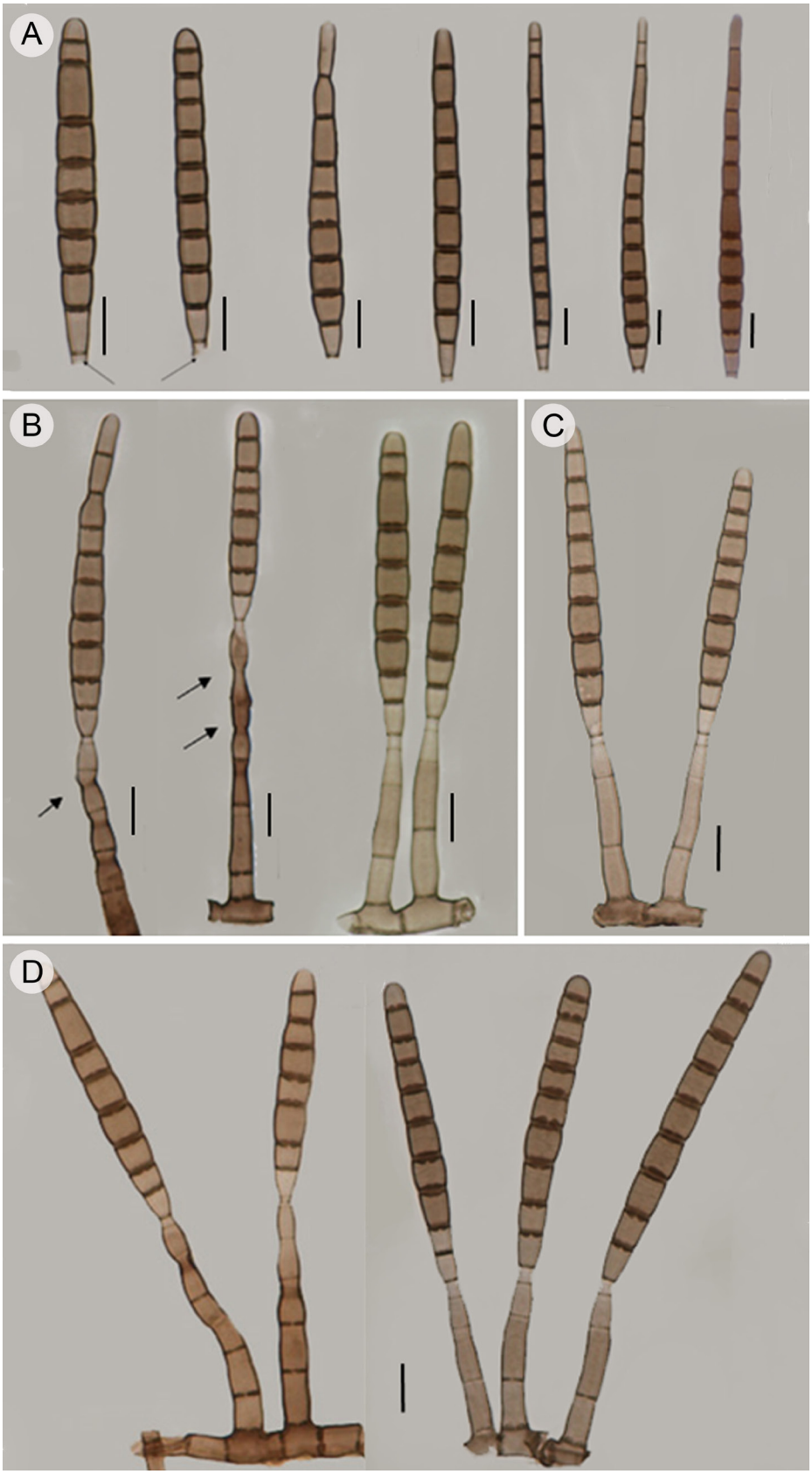

Figure 4: *Endophragmiella multiseptata* R.F. Castañeda, Heredia & R. M. Arias. A. conidia with basal frills (arrows); B. conidia attached to conidiophores with percurrent extensions (arrows); C-D. conidiophores in groups with conidia attached. Scale bars=10 µm. Photos by G. Heredia of *G. Heredia CB2304* (XAL).

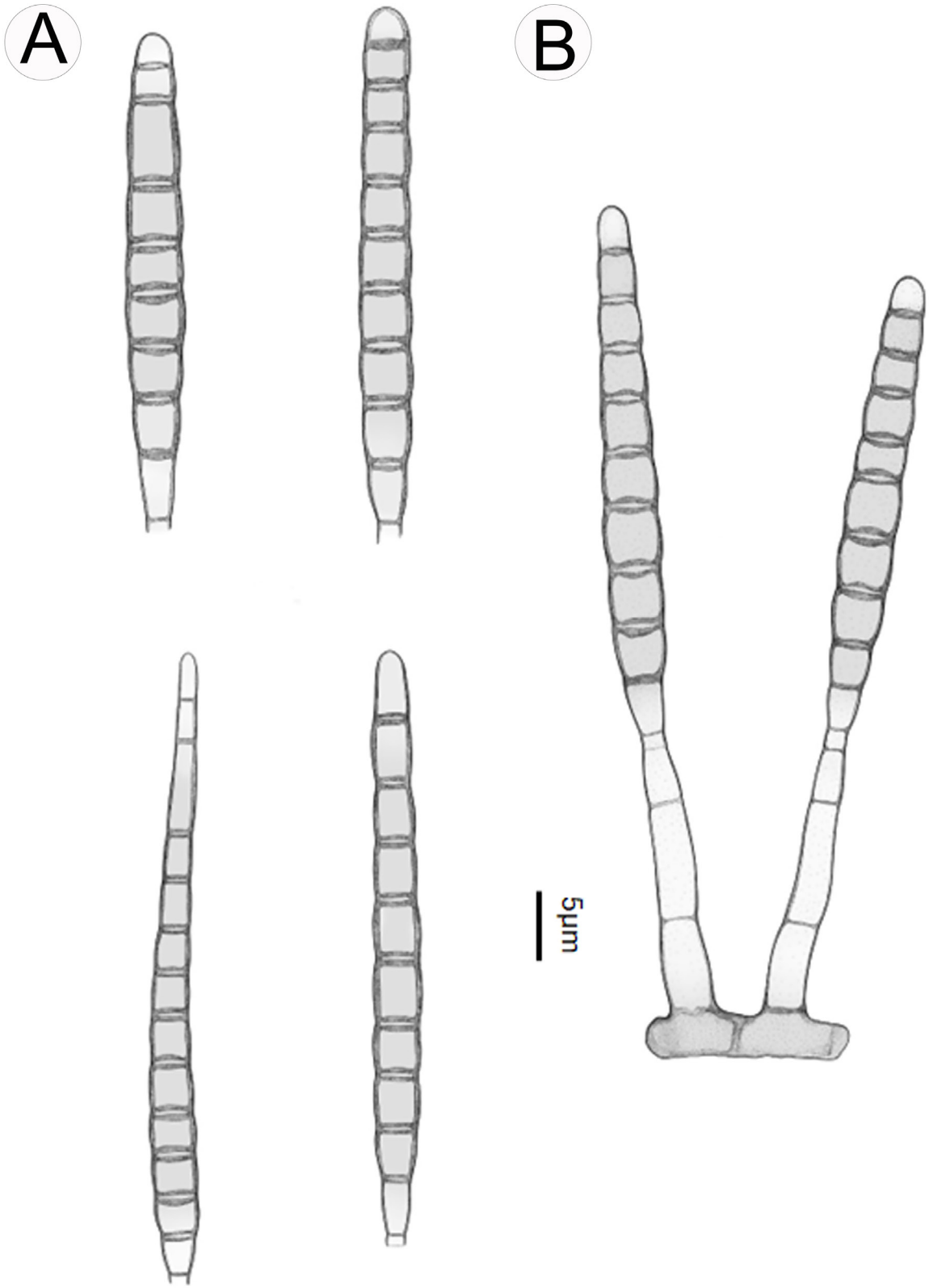

Figure 5: Line drawings of *Endophragmiella multiseptata* R.F. Castañeda, Heredia & R.M. Arias. A. conidia; B. conidiophores with conidia attached. Scale bars=10 µm. Drawn by María Teresa Jiménez Segura based on the holotype.

3-5-septate, smooth, brown below, paler brown near the apex, 30-62(-72.8) × 4-6.8 µm; conidiogenous cells monoblastic, integrated, terminal, cylindrical, attenuated and truncate at the apex, smooth, brown to pale brown, with 3-5 annellated, enteroblastic percurrent extensions; conidial secession rhexolytic; conidia solitary, acrogenous cylindrical to long obclavate, conico-truncate at base, rounded at the apex, (8-)9(-12)-euseptate, concolorous, pale brown, smooth, (48.5-)58-107(-120) long × 5-7.7(-8.2) µm wide ($\bar{x}$ = 75.6 × 6.8 µm, n=36),with a distinct basal frill of 2-2.5 µm long; synanamorph not observed.

Sexual morph: unknown.

Habit and habitat: saprobic, growing on decomposing fallen leaves in an oak-pine forest.

Etymology: the species epithet means having many septa in Latin.

Taxonomic notes: a comparison of *Endophragmiella* species having phragmoconidia with more than four eusepta is summarized in Table 2. Conidial shape, dimensions, and number of septa in *E. multiseptata* clearly differ from the remaining *Endophragmiella* species described so far. On the other hand, although *E. multiseptata* resembles morphologically some species of *Camposporium* Harkn. with cylindrical, pigmented phragmoconidia which may or may not bear appendages, e.g. *C. indicum* P. Rag. Rao & D. Rao (Rao and Rao, 1964), *C. ontariense* Matsush. (Matsushima, 1983), *C. cambrense* S. Hughes, or *C. pellucidum* (Grove) S. Hughes (Hughes, 1951), their denticulate (mono- or polyblastic) conidiogenous cells proliferating sympodially

Table 2: Comparison of the conidial morphology of *Endophragmiella* spp. with phragmoconidia similar to *Endophragmiella multiseptata* R.F. Castañeda, Heredia & R.M. Arias. The new species is highlighted in bold.

Taxon	Conidial characteristics				
	Shape	Septation	Pigmentation	Dimensions (µm)	Reference
Endophragmiella fagicola P.M. Kirk	Broadly fusiform to ellipsoid, obtuse at the apex, truncate at base	5-euseptate	Versicolorous, central cells brown to dark brown, basal and apical cells brown to pale brown	70-90 × 11-17	Kirk, 1981
Endophragmiella fusiformis W.P. Wu	Fusiform, apex rounded, often with a long, hyaline appendage	6-7-euseptate	Versicolorous, central cells brown to dark brown, basal and apical cells brown to pale brown	62-80 × 7.5-9	Wu & Zhuang, 2005
***Endophragmiella multiseptata* R.F. Castañeda, Heredia & R.M. Arias**	**Cylindrical to long obclavate, rounded at the apex**	**8-12(mostly 9)-euseptate**	**Concolorous, pale brown**	**(48.5-)58-107(-120) × 5-7.7(-8.2)**	**This study**
Endophragmiella profusa R.F. Castañeda, M. Stadler & Gené	Obovoid, clavate pyriform, obtuse at the apex, conical-truncate at base	(2-)3-5(mostly 5)-euseptate	Versicolorous, dark brown, with the basal cell pale brown to subhyaline	22-35 × 7-9	Castañeda Ruiz et al., 2010
Endophragmiella tenuis R.F. Castañeda	Obovoid, clavate, obtuse at the apex, conical-truncate at base	3-6(mostly 4)-euseptate	Versicolorous, dark brown, with basal and apical cell pale brown to subhyaline	37-50 × 5-9	Castañeda Ruiz, 1987

clearly differentiate them from species currently accepted within *Endophragmiella*.

Endophragmiella gardeniae Jian Ma & X.G. Zhang. Mycotaxon 119: 103-107. 2012. Fig. 6.

TYPE: CHINA. Hainan province, Hainan Bawangling National Nature Reserve, tropical forest, 11.XII.2009, *J. Ma* (holotype: HSAUP H5149; isotype: HMAS 146102).

Asexual morph, colonies on the natural substrate effuse, hairy, brown; mycelium partly immersed, composed of branched, septate, pale brown, smooth-walled hyphae, 1-2 µm wide; conidiophores macronematous, mononematous, solitary or in small groups, simple, erect, straight, or flexuous, up to 9-septate, smooth, pale brown to brown, up to 120 µm, 4-6.5 µm wide; with percurrent extensions; conidiogenous cells monoblastic, integrated, terminal, cylindrical, attenuated at the apex, smooth, pale brown, with 2-4 annellated enteroblastic percurrent extensions; conidial secession rhexolytic; conidia solitary, acrogenous, fusiform or obclavate, conico-truncate at base, 4-6-euseptate, slightly constricted at the septa, versicolorous, apical and basal cells pale brown to subhyaline, smooth, 39-53 µm long × 8.5-9.2 µm wide × 2.4-4 µm at the truncate base, with a distinct basal frill of 1-1.5 µm long; synanamorph selenosporella-like in the apical and subapical cells with conidiogenous cells up to 8 µm long × 1.6-3.7 µm wide and conidia filiform, acerose, aseptate, hyaline, up to 7.2 µm long × 0.4-0.9 µm wide.

Sexual morph: unknown.

Habit and habitat: saprobic, growing on rotten wood in a cloud forest.

Distribution: this species had not been further recorded since it was described by Ma and Zhang (Ma et al., 2012) on dead branches of *Gardenia hainanensis* Merr. from a tropical forest in China.

Examined material: MEXICO. Veracruz, municipality Acajete, Mesa de la Yerba, 1933 m, 19°33'40''N, 97°01'04''W, on rotten wood, 05.XII.2010, *G. Heredia and R. Arias CB1817* (XAL).

Taxonomic notes: the Mexican material agrees well with the holotype from China in shape, septation and size, as well as the disposition and shape of the selenosporella-like synanamorph. However, in our specimen the pigmentation of the conidial basal cell is paler (pale brown to subhyaline) than illustrated by Ma et al. (2012), and the apical branches are not that prominent, which could be attributed to the maturity state of the conidium or to intraspecific variability.

Discussion

Current phylogenetic status of *Endophragmiella*

The difficulty of growing *Endophragmiella* species in culture media has been pointed out by Chuaseeharonnachai et al. (2022). This problem, combined with the scarce recoverable fungal biomass from natural substrates, limits the application of molecular techniques to explore the phylogeny of the genus. So far in GenBank (2024), there are only sequences for the species *E. boewei* (ITS, LSU), *E. constricta* M.T. Dunn (ITS), *E. dimorphospora* Awao & Udagawa ex P.M. Kirk (ITS, LSU, SSU) and *E. taxi* M.B. Ellis ex P.M. Kirk (ITS, LSU).

Of the more than 100 described species in the genus, only *E. dimorphospora* has been the subject of phylogenetic studies. According to Hernández-Restrepo et al. (2017), based on molecular data from a soil strain (strain FMR 12150), the species is placed within the family Helminthosphaeriaceae in Sordariales (Sordariomycetes). This finding agrees well with Hughes (1979), who associated *E. biseptata* (Peck) S. Hughes with *Hilberina punctata* (Munk) A.N. Mill & Huhndorf (as *Lasiosphaeria punctata* Munk in Hughes' publication), a sexual morph also in Helminthosphaeriaceae. Considering the scarce molecular information for most species of *Endophragmiella,* it is probably too early to generalize their phylogenetic affinities. To overcome this problem, it is necessary to intensify the practice of adding alternative methods and culture media to obtain strains for sequencing.

On the other hand, considering the characteristics of the synanamorph, *E. chiapanensis* and *E. gardeniae* can

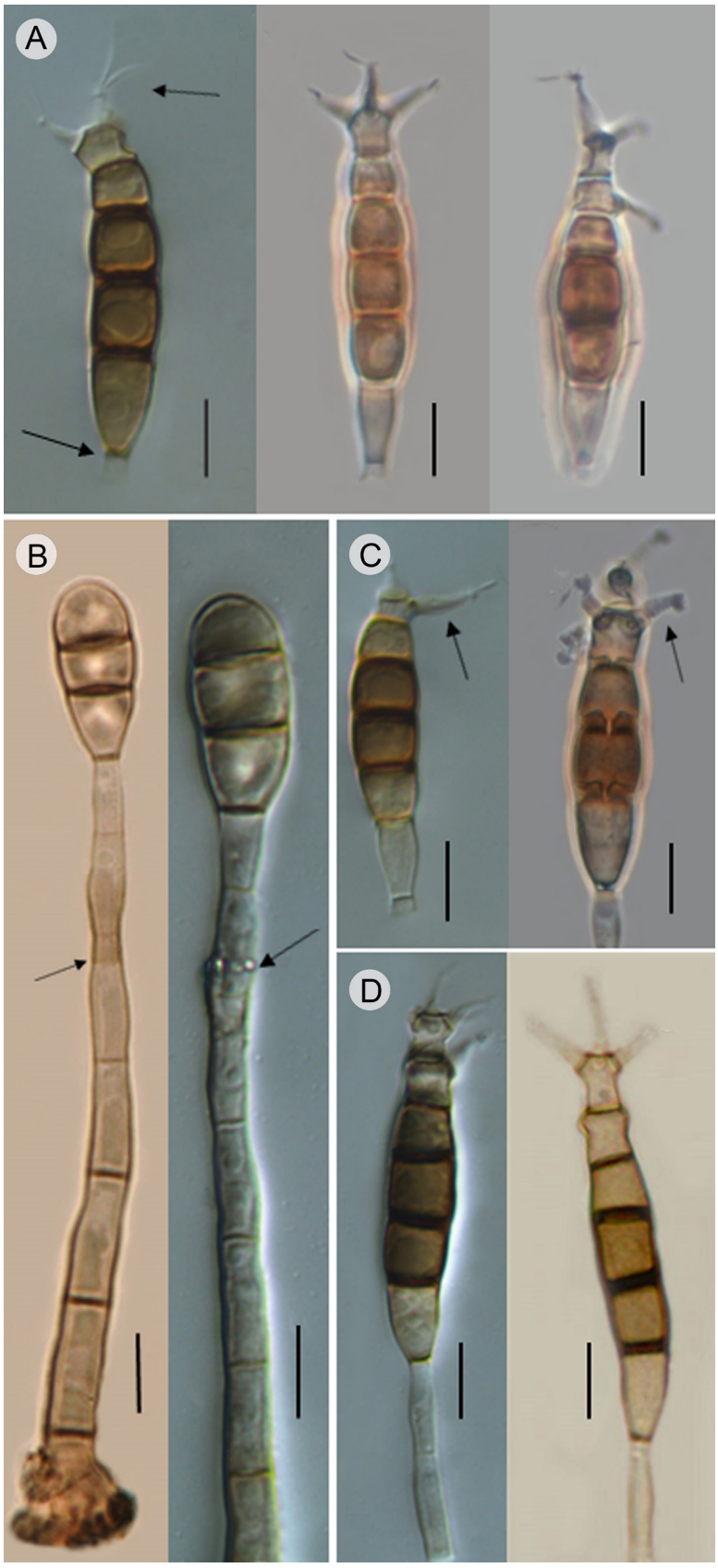

Figure 6: *Endophragmiella gardeniae* Jian Ma & X.G. Zhang. A. mature conidia with basal frills and apical spores of a selenosporella-like synanamorph (arrow); B. conidiophores with immature conidia showing percurrent extensions (arrows); C. conidia with conidiogenous cells of a selenosporella -like synanamorph (arrows); D. mature conidia attached to conidiogenous cells. Scale bars=10 µm. Photos by R. M. Arias of *G. Heredia and R. Arias CB1817* (XAL).

be considered pleo-anamorphic species with conidia of different types in morphology and conidiogenesis (Carmichel, 1981). Within *Endophragmiella,* selenosporella-like synanamorphs may arise directly from hyphae as in *E. theobromae* M.B. Ellis (Hughes, 1979), from conidiophores as in *E. lauri* P.M. Kirk & C.M. Kirk (Kirk, 1982a) and *E. africana* P.M. Kirk (Kirk, 1982b), or from conidia as in *E. acuta* (Wu and Zhuang, 2005), *E. machili* Jian Ma & X.G. Zhang (Ma et al., 2012), *E. selenosporellaria* (Ma et al., 2015), *E. theobromae*, *E. variabilis* R.F. Castañeda (Castañeda Ruiz, 1988), as well as in *E. chiapanensis* and *E. gardeniae*. Synanamorphs play important roles in the survival strategies of microfungi as propagative stages adapted to dispersal by air currents, rain, and insects (Carmichel, 1981). The mechanisms related to the induction of pleomorphism in these fungi have been scarcely studied (Wang, 1979), but it is very likely that the synanamorphs may play an important role in the evolution of these fungi.

The genus *Endophragmiella* in Mexico

Before this contribution 11 species of *Endophragmiella* had been recorded for Mexico, including *E. valdiviana,* recently transferred to *Camposporium* as *C. valdivianum* (Koukol and Delgado, 2021). Only *E. mexicana* J. Mena, Heredia & Mercado, collected on decaying oak leaves from a cloud forest in the state of Veracruz, had been described as a new species (Mercado Sierra et al., 1995). Besides *E. mexicana,* the following species were recorded as saprobic on plant debris from different localities in the state of Veracruz: *E. angustispora* S. Hughes and *E. boewei* (Arias et al., 2018), *E. cesatii* (Heredia Abarca et al., 2013), *E. collapsa* B. Sutton ex P.M. Kirk (Arias et al., 2010), *E. dimorphospora* (Heredia et al., 1997), *E. novae-zelandiae* S. Hughes (Arias et al., 2018), and *E. uniseptata* M.B. Ellis ex P.M. Kirk (Arias et al., 2018). Moreover, *E. pallescens* was detected on dead wood in a rainforest of Tabasco (Becerra Hernández et al., 2008) and *E. oblonga* Matsush. ex S. Hughes on oak leaves in an oak forest from the State of Mexico (Delgado-Zúñiga, 2020).

Given the great diversity of ecosystems in Mexico, the richness of *Endophragmiella* species must be considerably higher than what we currently know. However, compared with other Neotropical areas, Mexico should be considered probably the most extensively studied country in terms of *Endophragmiella* diversity.

Hereafter, we present an identification key to the species of the genus *Endophragmiella* in Mexico.

Key to the *Endophragmiella* species known in Mexico

1a. Conidia ellipsoidal, ovoid or obovoid 2
1b. Conidia differently shaped 8
2a. Conidia 1-euseptate 3
2b. Conidia 2-4-euseptate 5
3a. Conidia mostly versicolorous, brown to dark brown, with the lower cell paler than the apical one; 13-27 × 9-13 µm ..... *E. uniseptata* M.B. Ellis ex P.M. Kirk
3b. Conidia concolorous 4
4a. Conidia narrowly ovoid, pale brown to brown, mostly 1-euseptate, sometimes 2-septate; 14.4-20.5 × 4.5-5.4 µm ..... *E. angustispora* S. Hughes
4b. Conidia ellipsoidal, obtuse at the apex, oblong rounded at the ends, pale brown; 15-24 × 7-8 µm ..... *E. pallescens* B. Sutton
5a. Conidia 2-euseptate 6
5b. Conidia 3 or 4-euseptate 7
6a. Conidia with the apical cells thick-walled and dark brown, the basal cell pale brown; 14-17 × 7.2-8.3 µm ..... *E. collapsa* B. Sutton ex P.M. Kirk
6b. Conidia with the central cell darker than apical and basal cells; 19-28 × 9.5-11.5 µm ..... *E. oblonga* Matsush. ex S. Hughes
7a. Conidia mostly 3-euseptate, with the basal cell paler; 27-40 × 9.3-12.6 µm ..... *E. novae-zelandiae* S. Hughes
7b. Conidia up to 4-septate, often with broad black bands at the septa, with basal and distal cells subhyaline; 11-16 × 5.8-7.8 µm ..... *E. mexicana* J. Mena, Heredia & Mercado
8a. Conidia pyriform or fusiform 9
8b. Conidia cylindrical to long obclavate, rounded at the apex, 8-12-euseptate (mostly 9-euseptate), concolorous, light brown; (48.5-)58-107(-120) × 5-7.7(-8.2) µm ..... *E. multiseptata* R.F. Castañeda, Heredia & R. M. Arias

9a. Conidia pyriform 10
9b. Conidia fusiform 11
10a. Conidia versicolorous, apical cell dark brown to black, thick-walled, lower cell smaller, subhyaline; 18-22 × 11.7-14 µm .... *E. dimorphospora* Awao & Udagawa ex P.M. Kirk
10b. Conidia concolorous, subhyaline to light brown, 1-euseptate, lower cell smaller, truncate; 13.8-20.8 × 7.7-11.5 µm ..... *E. boewei* J.L. Crane ex S. Hughes
11a. Conidia mostly 3-euseptate 12
11b. Conidia mostly 5-euseptate, basal and apical cells subhyaline to hyaline; 39-55 × 8.5-9.2, with small branches bearing selenosporella-like synanamorph in the apex ..... *E. gardeniae* Jian Ma & X.G. Zhang
12a. Conidia with the central cells brown to deep brown, basal and apical cells subhyaline; 22-44 × 9-13 µm .... *E. cesatii* (Mont.) S. Hughes
12b. Conidia with central cells brown, basal and apical cells hyaline; (26.9-)30-36(-39.6) × (6.4-)6.8-8.5 (9) µm, often bearing selenosporella-like synanamorph on the apical cell of the conidia ..... *E. chiapanensis* R. F. Castañeda, Heredia & R. M. Arias

Conclusions

The new taxa *Endophragmiella chiapanensis* and *E. multiseptata* fit well with the generic concept of *Endophragmiella*, and they have morphological characteristics and dimensions that clearly distinguish them from the rest of the species accepted in the genus. With these new taxa, and the new record of *E. gardeniae*, a total of 13 *Endophragmiella* species are registered for Mexico.

Author contributions

All authors conceived and designed the study and contributed to the discussion, review, and approval of the final manuscript. GH and RMA collected the material, took the photos and elaborated all the plates.

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