Revista Mexicana de Biodiversidad

Revista Mexicana de Biodiversidad 97 (2026): e975566

Taxonomy and systematics

Epicoccum zapotae: a new species isolated from *Manilkara zapota* fruits in Central Mexico

Epicoccum zapotae*: una especie nueva aislada de frutos de* Manilkara zapota *en el centro de México*

Limni Silday Ramírez-Gallegos [a], Laura Navarro-de la Fuente [b], Ángel Trigos [b], Irene Lagunes [b, *]

[a] *Universidad Veracruzana, Centro de Investigación en Micología Aplicada, Doctorado en Micología aplicada, Calle Médicos 5, U.H. Del Bosque, 91010 Xalapa, Veracruz, Mexico*

[b] *Universidad Veracruzana, Centro de Investigación en Micología Aplicada, Calle Médicos 5, U.H. Del Bosque, 91010 Xalapa, Veracruz, Mexico*

*Corresponding author: roslagunes@uv.mx (I. Lagunes)

Received: 07 October 2024; accepted: 03 November 2025

Abstract

The genus *Epicoccum* exhibits substantial intraspecific morphological and genetic diversity, which has made it difficult to correctly identify and delimit species on the basis of morphological characteristics alone. Against this background, this study was aimed to report the morphological and molecular identification of a new species belonging to the genus *Epicoccum*, isolated from sapodilla fruit (*Manilkara zapota*) in Veracruz, Mexico, named *E. zapotae*. The morphological characteristics and the phylogenetic analysis of the concatenated sequences of LSU, ITS, and β-TUB support the fact that the isolate is a new species of the genus *Epicoccum*. This discovery contributes to the knowledge of the diversity of *Epicoccum* species in tropical ecosystems.

Keywords: Didymellaceae; *Epicoccum*; Morphology; Phylogenetic; Sapodilla fruit

Resumen

El género *Epicoccum* presenta diversidad morfológica y genética intraespecífica considerable, lo cual ha dificultado la correcta identificación y delimitación de especies con base únicamente en características morfológicas. En este contexto, el objetivo de este estudio fue reportar la identificación morfológica y molecular de una especie nueva perteneciente al género *Epicoccum*, aislada del fruto chicozapote (*Manilkara zapota*) en Veracruz, México, denominada *Epicoccum zapotae*. Las características morfológicas y el análisis filogenético de las secuencias

ISSN versión electrónica: 2007-8706; Universidad Nacional Autónoma de México, Instituto de Biología. https://doi.org/10.22201/ib.20078706e.2026.97.5566

concatenadas de LSU, ITS y β-TUB respaldan el hecho de que el aislado es una nueva especie del género *Epicoccum*. Este descubrimiento contribuye al conocimiento de la diversidad de especies de *Epicoccum* en ecosistemas tropicales.

Palabras clave: Didymellaceae; *Epicoccum*; Morfología; Filogenética; Fruto del chicozapote

Introduction

The genus *Epicoccum* belongs to the Didymellaceae family. It exhibits substantial intraspecific morphological and genetic diversity. It can be found in the air, soil, water, and various parts of plants including flowers, branches, leaves, and bark (Chen et al., 2017; Fávaro et al., 2011; Lee et al., 2020; Li et al., 2022). More than 70 species of the genus *Epicoccum* have been described, of which 5 species (*E. dendrobii*, *E. layuense*, *E. mezzettii*, *E. minitans*, *and E. nigrum*) have demonstrated biocontrol activity against phytopathogens (Braga et al., 2018; Taguiam et al., 2021). Species of this genus may exhibit a saprophytic (Braga et al., 2018), phytopathogenic (*E. nigrum* and *E. sorghinum*) (Chen et al., 2017; Taguiam et al., 2021) or endophytic lifestyle, the latter having isolated compounds with antioxidant, anticancer and antimicrobial activities (Braga et al., 2018; Taguiam et al., 2021).

The genus *Epicoccum* was originally established by Link (1816) based on observations of sporodochia in dry plant stems, with the following diagnosis: compact globose stroma dotted with subglobose spores. Link (1816) described the type species *E. nigrum*, however, his diagnosis was unsatisfactory and generated confusion (Schol-Schwarz, 1959). Subsequently, many species belonging to this genus were described, based on the fungus characteristics in its natural habitat, and therefore, they were named of the plant species from which the specimen was isolated (Schol-Schwarz, 1959).

Schol-Schwarz (1959) conducted a thorough review of the *Epicoccum* genus, examining 70 specimens displaying a variety of hues, including red, yellow, or olive, alongside an additional 96 specimens from herbaria around the world. Despite this extensive examination, an incomplete diagnosis was noted with descriptions that were primarily based on the fungus's original habitat characteristics and lacking the spore size measurements. For this reason, Schol-Schwarz (1959) considered the genus misclassified, concluding that color could not be used as a reliable taxonomic character, due to its susceptibility to various abiotic factors such as medium, light, pH, etc.; thus, no valid basis remained for splitting the genus into separate species, despite its wide range of conidial dimensions and cultural characters. Consequently, the entire genus was reduced to the single variable species *E. nigrum* (Kilpatrick & Chilvers, 1981).

Noting the lack of molecular evidence to support Schol-Schwarz´s classification, Wang and Guo (2004) performed the molecular identification of 45 *E. nigrum* isolates by amplifying the 5.8S gene and flanking internal transcribed spacer regions (ITS1 and ITS2) of the nuclear ribosomal DNA. They found that *E. nigrum* could comprise more than 1 species, ultimately concluding that conidial size and colony color should not be used as reliable taxonomic characteristics in the identification of *E. nigrum*. Later, Fávaro et al. (2011) acknowledged the need to reevaluate the classification of *E. nigrum* as a single variable species and many sequences deposited as *E. nigrum* in the GenBank database and fungal strains cultures many of which should be reclassified. Accurate species identification has long been challenging due to its heavy reliance on host plant morphology and association (Chen et al., 2015).

Therefore, taxonomic studies based on multilocus phylogeny using LSU, ITS, rpb2 and tub2 sequences in combination with morphological differences, allowed revising the classification within the Didymellaceae family, including the genus *Epicoccum*, providing a relatively robust phylogenetic basis for taxon delineation (Aveskamp et al., 2010; Braga et al., 2018; Chen et al., 2015; Chen et al., 2017). Currently, 18 *Epicoccum* species are accepted with their correct morphological and molecular identification (Braga et al., 2018). In this context, this study was aimed to report the morphological and molecular identification of a new species belonging to the genus *Epicoccum*.

Materials and methods

Sapodilla fruits were collected during May-June 2017 from 10-12 tall *Manilkara zapota* trees in one ~ 1 ha orchard close to the village of Apazapan, Veracruz, Mexico. Fruit samples were transported to the laboratory at the Centro de Investigación en Micología Aplicada (CIMA), Universidad Veracruzana. The samples were washed with running tap water to remove residual soil, afterwards the fruits were cut into small pieces (0.5 cm^2), disinfected with 2% sodium hypochlorite for 30 seconds, and washed twice with sterile water. The disinfected samples were transferred onto Petri dishes containing Potato Dextrose Agar (PDA) medium (MCD LAB, San Jacinto Amilpas, Oax, MX) with 0.2 g/L chloramphenicol. The Petri dishes

were incubated in dark conditions at 25 ± 2 °C for 2-3 days until mycelial growth was developed from the samples. Newly developed mycelia were immediately transferred to fresh PDA plates and incubated under the same conditions for fungal isolation. To ensure the purity of the fungal isolate, monosporic cultures were developed by cutting hyphal tips under a stereoscopic microscope (Leica EZ4), transferred to PDA plates and incubated under the same conditions established.

Culture characteristics were determined after 14 days of cultivation at 25 ± 2 °C on PDA. Colony diameters were measured after 7 days in incubation. The hyphae and reproductive structures produced on PDA were morphologically identified using an optical microscope at 100 × magnification. The mycelium was mounted on a microscope slide with a drop of lactophenol blue solution; the diameter of the conidia was measured (n = 100), means and standard errors (SE) were calculated, with extreme values shown in parentheses. The reproductive structures were identified using the taxonomic keys reported by Barnett and Hunter (1972). The holotype specimen (CM-CNRG 1003, dried agar plate culture) was deposited in the Microorganism Collection of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) in Mexico.

Genomic DNA was extracted from 14 days old mycelium grown on PDA according to Liu et al. (2000). The internal transcribed spacer region (ITS) of the nuclear ribosomal DNA (nrDNA) gene, the partial large subunit (LSU) nrDNA gene, and the β-tubulin (TUB) gene were amplified. PCR was performed using the primers listed in Table 1. DNA was amplified in a SureCycler 8800 thermal cycler with the same conditions for each gene, only the annealing temperature varied. The reaction mixture was incubated as follows: initial denaturation at 94 °C for 3 min, followed by 34 cycles at 94 °C for 1 min, the annealing temperatures are shown in Table 1, extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. The amplifications were purified using the Wizard® SV Gel and PCR System Clean-Up kit and sent to Labsergen Langebio (Cinvestav, Irapuato, Gto., Mexico) for sequencing on an AB3770 capillary sequencer.

Consensus sequences were created with BioEdit software (Hall, 1999) and were compared in the GenBank nucleotide sequence database (Benson et al., 2017) using BLAST search software (Zhang et al., 2000) to confirm the genus and percentage of identity. Closely related species sequences and our newly obtained sequences (Table 2) were incorporated into sequence datasets independently for each molecular marker using PhyDE v.0.9971 Phylogenetic Development (Müller et al., 2010). Each dataset was independently aligned using the MAFFT online service (Katoh et al., 2019). Inconsistencies were manually adjusted using the MESQUITE 3.61 phylogenetic data editor (Maddison & Maddison, 2019), the same program with which the concatenated ITS + LSU + TUB sequences dataset were integrated. The GTR + G nucleotide substitution model was selected using the jModelTest v.2.1.4 program (Darriba et al., 2012) according to the Akaike Information Criterion (AIC). The concatenated aligned dataset was analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analyses were conducted with RAxMLGUI2.0 (Edler et al., 2020) using 1,000 bootstrap replicates, the robustness of the analyses was evaluated by bootstrap support (BS) values. Bayesian Inference analyses were performed on MrBayes v.3.2.1 software, using the substitution model selected for each matrix by jModelTest, based on the Akaike Information Criterion (AIC) (Table 3), with 4 Markov Chains Monte Carlo (MCMC) and 1 million generations (Ronquist et al., 2012). The ITS, LSU and TUB sequences generated in this study were deposited in the GenBank database under the accession numbers provided in the taxonomic section.

The Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model (Taylor et al., 2000) was used to delimit the fungal species and analyze phylogenetic relationships among closely related taxa. Recombination levels within these phylogenetically proximate species

Table 1
Primers and annealing temperatures used in the PCR amplifications step.

Locus	Primer	Primer DNA sequences (5′–3′)	Annealing T (°C) / Time (s)	Reference
ITS	ITS1F	CTTGGTCATTTAGAGGAAGTAA	53/45	Gardes & Bruns, 1993; White et al., 1990
	ITS4	TCCTCCGCTTATTGATATGC		
LSU	LR0R	ACCCGCTGAACTTAAGC	55/60	Vilgalys & Hester, 1990
	LR7	TACTACCACCAAGATCT		
TUB	TUB1	AACATGCGTGAGATTGTAAGT	57/60	Woudenberg et al., 2009
	TUB22	TCTGGATGTTGTTGGGAATCC		

Table 2
Species and GenBank accession number of sequences used for the construction of the phylogenetic tree.

Species	Voucher	Country	GenBank accession number		
			ITS	LSU	TUB
E. brahmansense	CBS:990.95	Papua New Guinea	MN973514	MN943720	MT005614
E. brahmansense	CBS 985.95	Papua New Guinea	MN973513	MN943719	MT005613
E. camelliae	LC:4862	China	KY742092	KY742246	KY742334
E. dendrobii	LC:8145 [T]	China	KY742093	KY742247	KY742335
E. draconis	CBS 186.83	Rwanda	GU237795	GU238070	GU237607
E. duchesneae	CBS 218.81	India	MN972935	MN973322	MN983950
E. duchesneae	LC:5139 [T]	China	KY742095	KY742249	KY742337
E. hordei	LC:8149	Australia	KY742098	KY742252	KY742340
E. hordei	LC:8148 [T]	Australia	KY742097	KY742251	KY742339
E. huancayense	CBS:105.80	Peru	MH861244	MH873016	GU237615
E. huancayense	CBS 390.93	Peru	GU237857	GU238085	GU237616
E. layuense	LC:8155 [T]	China	KY742107	KY742261	KY742349
E. mezzettii	CBS 173.38	Italy	MN973496	MN943701	MT005596
E. mezzettii	CBS 238.59	No data	MN973494	MN943699	MT005594
E. multiceps	CBS:119734	Brazil	MN973512	MN943718	MT005612
E. nigrum	CBS 173.73	USA	FJ426996	GU237975	FJ427107
E. nigrum	CBS 125.82	Netherlands	FJ426995	GU237974	FJ427106
E. oryzae	CBS:174.34	Japan	MN973500	MN943705	MT005600
E. oryzae	CBS:173.34	Japan	MN973499	MN943704	MT005599
E. ovisporum	CBS 180.80 [T]	No data	NR158228	NG069492	FJ427174
E. plurivorum	CBS 558.81	New Zealand	GU237888	GU238132	GU237647
E. poaceicola	CBS:987.95	Papua New Guinea	MN972955	MN973343	MN983969
E. poae	LC:8161	USA	KY742114	KY742268	KY742356
E. poae	LC:8160 [T]	USA	KY742113	KY742267	KY742355
E. proteae	CBS:114179 [T]	South Africa	MH862956	MH874519	LT623230
E. tobaicum	JCK-CSHF10	South Korea	MW368670	MW368670	MW392085
E. variabile	CBS:119733	Brazil	MN973501	MN943706	MT005601
E. viticis	CGMCC 3.18344 [T]	China	NR158267	NG069447	KY742360
E. zapotae	CM-CNRG 1003	Mexico	OQ333010	OQ333009	OQ330858
D. americana	CBS 185.85	USA	FJ426972	GU237990	FJ427088

[T] Indicates type strains

were assessed through the calculation of the pairwise homoplasy index (PHI) using the SplitsTree4 software (Huson, 1998; Huson & Bryant, 2006). A concatenated dataset including ITS, LSU, and TUB was used for the analyses. Splits graphs were generated in SplitsTree4 utilizing both the Log-Det transformation and splits decomposition options, facilitating the visualization of the phylogenetic relationships among the species. Interpretation of a pairwise homoplasy index value falling below the 0.05 significance threshold ($\Phi w < 0.05$) was taken to signify the presence of recombination within the analyzed dataset.

Table 3

The nucleotide substitution model for each matrix obtained by jModelTest based on the Akaike Information Criterion.

Locus	Length	Substitution model	MrBayes parameters (Nst-rates)
ITS	455 pb	TIM2 + I + G	6-invgamma
LSU	765 pb	TrN + I + G	6-invgamma
TUB	330 pb	TIM3 + G	6-gamma

Description

Epicoccum zapotae L. Navarro & L. Ramírez sp. nov. (Fig. 1)

Diagnosis: dark sporodochia, conidia dark pigmented, globose or obovoid with radial separation and a verrucous outer surface. Compared to other species in the genus, colonies growth on PDA exhibits a distinctive pink pigmentation.

Sexual morph: not observed.

Asexual morph: Sporodochia are dark. Conidiophores are compact or loose, and light brown. Conidia dark pigmented, globose or obovoid with radial septation and a verrucous outer surface, with an average size 10.48 ± 1.64 µm (8.5-13.4 µm) (Fig. 2).

Culture characteristics: Colonies on PDA measured 33 ± 2 mm in diameter after 7 days, furrowed and circular with filamentous margin and flattened aerial mycelia. The central region of the colonies appears light pink, surrounded by concentric rings of intense pink coloration.

This strain was deposited at Colección de Microorganismos del Centro Nacional de Recursos Genéticos (CNRG), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Tepatitlán de Morelos, Jalisco, Mexico, under accession number CM-CNRG 1003.

Taxonomic summary

Mycobank number: MB 853124

GenBank: OQ333010 (ITS), OQ333009 (LSU), OQ330858 (B-TUB).

Type: Mexico, Veracruz State: Apazapan (19°20'17.16" N, 96°43'54.12" W, 300 m), isolated from the fruit of *M. zapota*, June 2017, Holotype, CM-CNRG 1003.

Etymology: *zapotae*, referring to the host plant species (*Manilkara zapota*) from which it was isolated.

Remarks

Phylogenetic tree constructed from concatenated ITS + LSU + TUB sequences of 30 different species, with a final length of 1,550 characters including gaps. Sequences of *Didymella americana* CBS 185.85 were selected as outgroup (Table 2, Fig. 3). Branches of the phylogenetic tree are labeled with their respective bootstrap values (BS) and the Bayesian posterior probabilities (BPP). Our consensus phylogenetic tree generated from the ML analysis with BS/BPP values robustly supports (BS = 98 / BPP = 1) the assignment of our isolate as an independent species within the *Epicoccum* genus, positioned as a sister taxon to *E. dendrobii* (Fig. 3).

Application of the GCPSR analysis to our dataset yielded a PHI value of 0.5502 (Fig. 4). This outcome suggests that there is no substantial evidence of recombination occurring between *E. zapotae* and its related taxa. These results indicate that the *E. zapotae* isolate is distinctly differentiated from *E. dendrobii* and there is no indication of recombination events between them.

The robustness of BS/BPP values 98/1 supports the placement of *E. zapotae* as a sister species to *E. dendrobii*. This relationship forms a distinct clade, positioned adjacent to *E. layuense*, *E. mezzettii*, *E. nigrum*, *E. oryzae*, *E. poae*, and *E. tobaicum*.

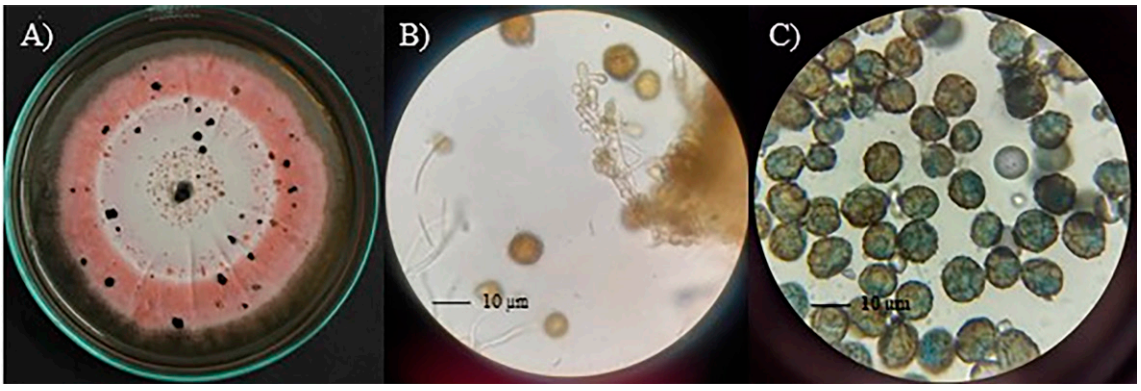

Figure 1. *E. zapotae* (CM-CNRG 1003). A) Colony on PDA with the presence of sporodochia, B) conidiophore 100 ×, C) conidia 100 ×.

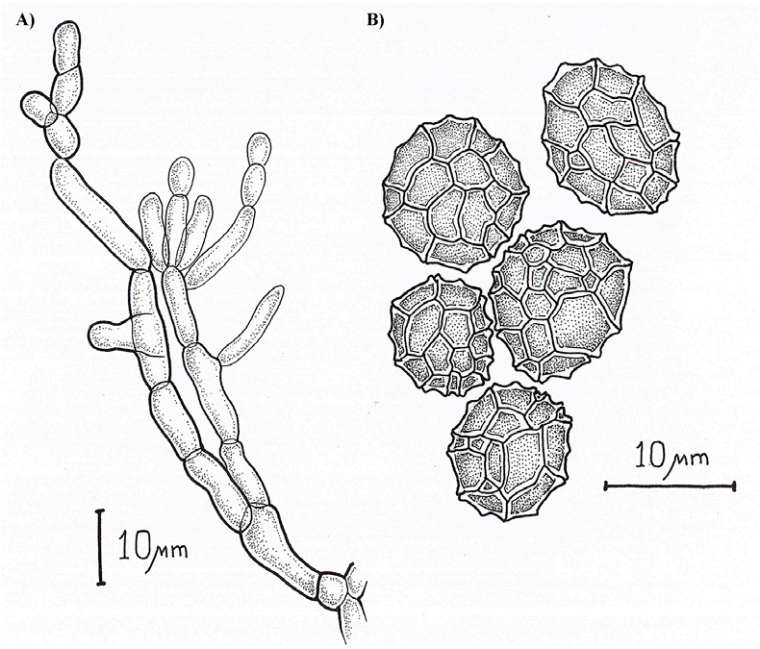

Figure 2. Schematic representation of the microscopic characteristics of *Epicoccum zapotae*. A) Conidiophore, B) conidia.

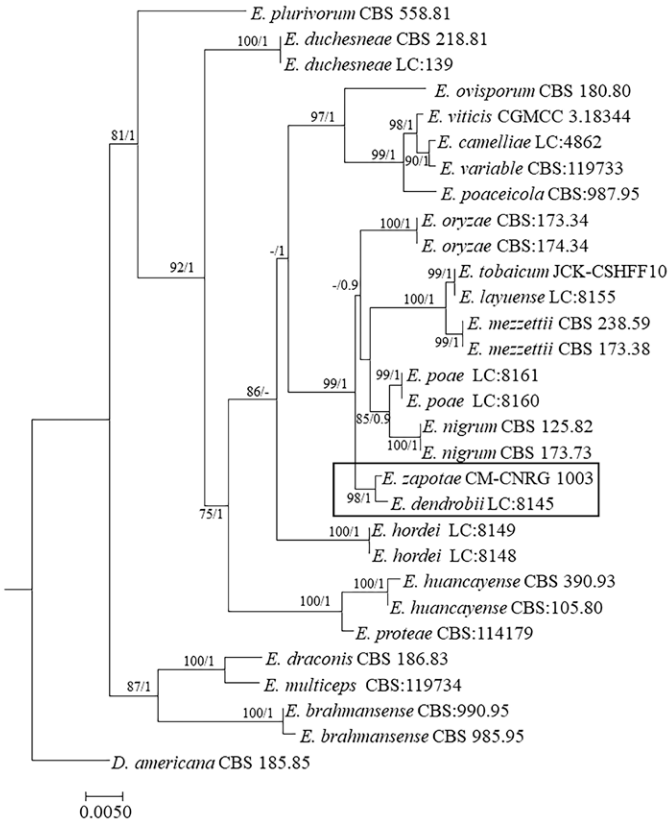

Figure 3. Maximum likelihood phylogenetic tree generated from the analysis of the concatenated ITS + LSU + TUB sequences of *E. zapotae* (holotype, CM-CNRG 1003) and species of the genus *Epicoccum* reported in the GenBank. The numbers at the nodes indicated Bootstrap values for 1,000 replicates (BS ≥ 70 %)/Bayesian Posterior Probabilities (BPP ≥ 0.9).

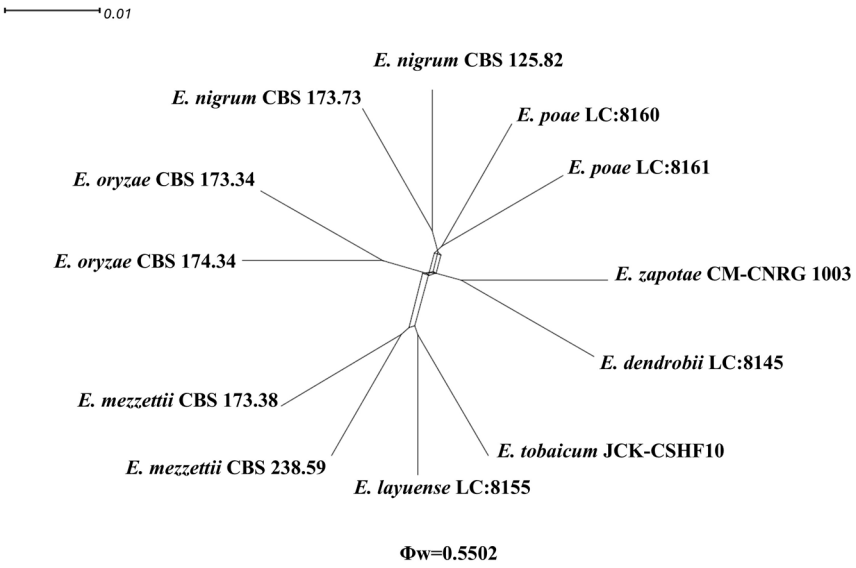

Figure 4. Results of the pairwise homoplasy index (PHI) test for *E. zapotae* and closely related species. PHI test results (φw) > 0.05 indicate no significant evidence of recombination within the analyzed dataset.

Epicoccum dendrobii exhibits the closest genetic proximity to *E. zapotae*. Although *E. zapotae* shares general morphological traits with other *Epicoccum* species, distinct features support the classification of *E. zapotae* as a separate species. Conidia of *E. dendrobii* are multicellular-phragmosporous, verrucose, subglobose-pyriform, brown, with a basal cell, 11-19 µm diam (Chen et al., 2017). In contrast, conidia of *E. zapotae* are smaller, ranging from 8.84-12.12 µm diam. Additionally, the radial growth rate of *E. dendrobii* on PDA at 25 °C reaches 34-38 mm diam after 7 days. There is also a significant difference in culture characteristics on PDA between the 2 species, colonies of *E. dendrobii* display a regular margin, with flat aerial mycelia felty to floccose in texture, white to buff in color, becoming olivaceous near the center (Chen et al., 2017). In contrast, colonies of *E. zapotae* show slower growth on PDA (31-35 mm) for 7 days and their colonies are distinguished by their intense pink color (Table 4).

Epicoccum dendrobii has been reported to cause black leaf spots on *Dendrobium fimbriatum* (Chen et al., 2017). However, *E. zapotae* was isolated from sapodilla fruits without visible disease symptoms; therefore, it was probably plant-associated as an endophytic fungus, that

Table 4

Comparative morphological characteristics between *E. dendrobii* and *E. zapotae*. Data for *E. dendrobii* adapted from Chen et al. (2017).

Characteristic	*E. dendrobii*	*E. zapotae*
Conidia	Multicellular-phragmosporous, verrucose, subglobose-pyriform, brown	Dark pigmented, globose to obovoid with radial separation and a verrucous outer surface
Conidial diameter	11-19 µm	8.84-12.12 µm
Colony margin (PDA)	Regular	Furrowed and circular
Aerial mycelium (PDA)	Felty to floccose	Flattened
Colony color (PDA)	Beige	Pink
Colony diameter (7 days)	34-38 mm	31-35 mm

can grow asymptomatically within plant tissues (Wen et al., 2022). The ability of endophytic fungi to produce bioactive compounds with biomedical applications has been widely explored (Hashem et al., 2023). In particular, from *Epicoccum* sp. known as an endophyte of *Theobroma cacao*, the bioactive compounds epicolactone, epicoccolide A and epicoccolide B have been isolated (Talontsi et al., 2013). Therefore, *E. zapotae* represents an opportunity for mycochemical studies aimed at exploring the biomedical potential of the bioactive compounds it produced.

Our phylogenetic and morphological analysis, as well as the results from the GCPSR analysis confirm that our isolate, despite its greater genetic proximity to *E. dendrobii*, presents distinct morphological differences ranging from the size and shape of the conidia to their growth on PDA. This demonstrates that our isolate represents a new species of the genus *Epicoccum*.

Our comprehensive morphological and molecular analyses have led us to conclude that fungal isolate recovered from the *M. zapota* fruit, is a new species within the genus *Epicoccum* which we report as *E. zapotae*. This discovery represents the first report of *E. zapotae* isolated from the sapodilla fruits in Veracruz, Mexico, and provides an opportunity for bioprospecting studies aimed at exploring its pharmaceutical potential.

Acknowledgements

This research was supported by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (Secihti), formerly Conahcyt (FORDECYT PRONACES CF/304020 project) and the Universidad Veracruzana (UV-CA-354). L.S.R-G thanks Secihti for the predoctoral grant 802758.

References

Aveskamp, M. M., de Gruyter, J., Woudenberg, J. H. C., Verkley, G. J. M., & Crous, P. W. (2010). Highlights of the Didymellaceae: a polyphasic approach to characterize *Phoma* and related pleosporalean genera. *Studies in Mycology*, *65*, 1–60. https://doi.org/10.3114/sim.2010.65.01

Barnett, H. L., & Hunter, B. B. (1972). *Illustrated genera of imperfect fungi. 3rd Ed.* Minneapolis: Burgess Publishing Company.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. et al. (2017). GenBank. *Nucleic Acids Research*, *45*, D37–D42. https://doi.org/10.1093/nar/gkw1070

Braga, R., Padilla, G., & Araújo, W. L. (2018). The biotechnological potential of *Epicoccum* spp.: diversity of secondary metabolites. *Critical Reviews in Microbiology*, *44*, 759–778. https://doi.org/10.1080/1040841X.2018.1514364

Chen, Q., Jiang, J. R., Zhang, G. Z., Cai, L., & Crous, P. W. (2015). Resolving the *Phoma* enigma. *Studies in Mycology*, *82*, 137–217. https://doi.org/10.1016/j.simyco.2015.10.003

Chen, Q., Hou, L. W., Duan, W. J., Crous, P. W., & Cai, L. (2017). Didymellaceae revisited. *Studies in Mycology*, *87*, 105–159. https://doi.org/10.1016/j.simyco.2017.06.002

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. https://doi.org/10.1038/nmeth.2109

Edler, D., Klein, J., Antonelli, A., & Silvestro, D. (2020). raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution*, *12*, 373–377. https://doi.org/10.1111/2041-210X.13512

Fávaro, L. C., de Melo, F. L., Aguilar-Vildoso, C. I., & Araújo, W. L. (2011). Polyphasic analysis of intraspecific diversity in *Epicoccum nigrum* warrants reclassification into separate species. *Plos One*, *6*, e14828. https://doi.org/10.1371/journal.pone.0014828

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series*, *41*, 95-98.

Hashem, A. H., Attia, M. S., Kandil, E. K., Fwazi, M. M., Abdelrahman, A. S., Khader, M. S. et al. (2023). Bioactive compounds and biomedical application of endophytic fungi: a recent review. *Microbial Cell Factories*, *22*, 107. https://doi.org/10.1186/s12934-023-02118-x

Huson, D. H. (1998). SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics*, *14*, 68–73. https://doi.org/10.1093/bioinformatics/14.1.68

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, *23*, 254–267. https://doi.org/10.1093/molbev/msj030

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*, 1160–1166. https://doi.org/10.1093/bib/bbx108

Kilpatrick, J. A., & Chilvers, G. A. (1981). Variation in a natural population of *Epicoccum purpurascens*. *Transactions of the British Mycological Society*, *77*, 497–508. https://doi.org/10.1016/S0007-1536(81)80097-6

Lee, A. J., Cadelis, M. M., Kim, S. H., Swift, S., Copp, B. R., & Villas-Boas, S. G. (2020). Epipyrone A, a broad-spectrum antifungal compound produced by *Epicoccum nigrum* ICMP 19927. *Molecules*, *25*, 5997. https://doi.org/10.3390/molecules25245997

Li, T., Im, J., & Lee, J. (2022). Genetic diversity of *Epicoccum nigrum* and its effects on *Fusarium graminearum*.

Mycobiology, *50*, 457–466. https://doi:10.1080/12298093.2022.2148394

Link, H. F. (1816). Observationes in ordines plantarum naturales III. *Magazin der Gesellschaft Naturforschender Freunde Berlin*, *7*, 37–38.

Liu, D., Coloe, S., Baird, R., & Pedersen, J. (2000). Rapid mini-preparation of fungal DNA for PCR. *Journal of Clinical Microbiology*, *38*, 471. https://doi:10.1128/jcm.38.1.471-471.2000

Maddison, W., & Maddison, D. (2019). Mesquite: a modular system for evolutionary analysis. Version 3.61. https://www.mesquiteproject.org

Müller, J., Müller, K., Neinhuis, C., & Quandt, D. (2010). PhyDE Phylogenetic data editor, version 0.9971. *Program distributed by the authors*. http://www.phyde.de

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S. et al. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Schol-Schwarz, M. B. (1959). The genus *Epicoccum* link. *Transactions of the British Mycological Society*, *42*, 149–173. https://doi.org/10.1016/S0007-1536(59)80024-3

Taguiam, J. D., Evallo, E., & Balendres, M. A. (2021). *Epicoccum* species: ubiquitous plant pathogens and effective biological control agents. *European Journal of Plant Pathology*, *159*, 713–725. https://doi.org/10.1007/s10658-021-02207-w

Talontsi, F. M., Dittrich, B., Schüffler, A., Sun, H., & Laatsch, H. (2013) Epicoccolides: antimicrobial and antifungal polyketides from an endophytic fungus *Epicoccum* sp. associated with *Theobroma cacao*. *European Journal of Organic Chemistry*, *2013*, 3174–3180. https://doi.org/10.1002/ejoc.201300146

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S. et al. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology*, *31*, 21–32. https://doi.org/10.1006/fgbi.2000.1228

Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology*, *172*, 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang, Y., & Guo, L. D. (2004). Morphological and molecular identification of an endophytic fungus *Epicoccum nigrum*. *Mycosystema*, *23*, 474–479. https://doi:10.3329/bipt.v29i2.63534

Wen, J., Okyere, S. K., Wang, S., Wang, J., Xie, L., Ran, Y. et al. (2022). Endophytic fungi: an effective alternative source of plant-derived bioactive compounds for pharmacological studies. *Journal of Fungi*, *8*, 205. https://doi.org/10.3390/jof8020205

White, T. J., Bruns, T. D., Lee, S. B., & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR Protocols: a guide to methods and applications* (pp. 315–322). New York: Academic Press. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Woudenberg, J. H. C., Aveskamp, M. M., de Gruyter, J., Spiers, A. G., & Crous, P. W. (2009). Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *22*, 56–62. https://doi.org/10.3767/003158509X427808

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, *7*, 203–214. https://doi.org/10.1089/10665270050081478