First report of *Apiospora intestini* (Apiosporaceae) in blackberry (*Rubus* sp., Rosaceae) in Mexico

Primer reporte de *Apiospora intestini* (Apiosporaceae) en zarzamora (*Rubus* sp., Rosaceae) en México

Edith Garay-Serrano[1,2,3] (iD) and Jazmin Cortes-Martinez[1] (iD)

Abstract:

Background and Aims: The genus *Apiospora* comprises 157 microscopic fungal species worldwide. It includes saprobes, symbiotic, endophytic and pathogens of both plants and humans. In a survey of phytopathogenic organisms of blackberry (*Rubus* sp.) stems in Taretan, Michoacán, Mexico, we isolated a fungus whose identification at genus level was unclear. The objectives of this study were to identify the isolate at species level and determine whether it represents or not a pathogenic organism for blackberry plants.

Methods: The fungal strain INE47 was isolated from the stems of *Rubus* 'Tupy'. DNA extraction was performed, and the sequences of the internal transcribed spacer (ITS) and translation elongation factor 1-alpha (TEF1-α) gene of the isolate were obtained. To construct the phylogenetic trees, *Apiospora*-related sequence matrices were analyzed for each marker separately and concatenated, using Bayesian Inference and Maximum Likelihood methods. Koch´s test were performed on blackberry stems and leaves to determine the pathogenic ability of the isolate. Finally, the morphological description of the colony and the micro morphological structures such as spores and conidiophores was completed.

Key results: INE47 was identified as *Apiospora intestini* hosted by blackberry plants in Michoacán, Mexico. After pathogenicity tests, the species caused a small spot in the inoculated area on blackberry stems and leaves. The disease severity induced for this isolate does not represent an important impact for this crop.

Conclusions: *Apiospora intestini* found on blackberry represents the first report of this fungus in Mexico. The pathogenicity test and the evaluation of the disease severity demonstrated that it is a weak pathogen on stem and leaves of blackberry, causing necrotic spots.

Key words: microscopical fungus, necrotic spots, phylogeny, plant pathogen.

Resumen:

Antecedentes y Objetivos: El género *Apiospora* comprende 157 especies de hongos microscópicos a nivel mundial. Incluye saprobios, simbióticos, endófitos y patógenos, tanto de plantas como de humanos. En un estudio de organismos fitopatógenos de tallos de zarzamora (*Rubus* sp.) en Taretan, Michoacán, México, aislamos un hongo cuya identificación a género era incierta. Los objetivos de este estudio fueron identificar el aislado a nivel de especie y determinar si representa o no un organismo patógeno para las plantas de zarzamora.

Métodos: Se aisló la cepa fúngica INE47 de los tallos de *Rubus* 'Tupy'. Se realizó la extracción del ADN y se obtuvieron las secuencias del espaciador transcrito interno (ITS) y del gen del factor de elongación de la traducción 1-alfa (TEF1-α) del aislado. Para construir los árboles filogenéticos, se analizaron las matrices de secuencias relacionadas con *Apiospora*, para cada marcador por separado y concatenados, usando los métodos de Inferencia Bayesiana y Máxima Verosimilitud. Se llevaron a cabo pruebas de Koch en tallos y hojas de zarzamora para determinar la habilidad patogénica del aislado. Finalmente, se realizó la descripción morfológica de la colonia y de las estructuras micro morfológicas como esporas y conidióforos.

Resultados clave: INE47 fue identificada como *Apiospora intestini* habitando en plantas de zarzamora en Michoacán, México. Tras las pruebas de patogenicidad, la especie causó una pequeña mancha necrótica en el área inoculada en los tallos y hojas de zarzamora. La severidad de la enfermedad inducida por este aislado no representa un impacto importante para este cultivo.

Conclusiones: *Apiospora intestini* encontrado en zarzamora representa el primer reporte de este hongo en México. La prueba de patogenicidad y la evaluación de la severidad de la enfermedad demostraron que es un patógeno débil, causando manchas necróticas en los tallos y hojas de zarzamora.

Palabras clave: filogenia, fitopatógeno, hongo microscópico, manchas necróticas.

[1]Instituto de Ecología, A.C., Centro Regional del Bajío, Red de Diversidad Biológica del Occidente Mexicano, Avenida Lázaro Cárdenas 253, 61600 Pátzcuaro, Michoacán, Mexico.
[2]Secretaría de Ciencia, Humanidades, Tecnología e Innovación, Avenida Insurgentes Sur 1582, 03940 Cd. Mx., Mexico.
[3]Author for correspondence: edith.garay@inecol.mx

Introduction

The species of the genus *Apiospora* Sacc. are a group of microscopic fungi of the family Apiosporaceae within the phylum Ascomycota. In Index Fungorum (2024), from 1875 to 2024, there are 182 records, which correspond to 157 recognized species in the genus.

Although the members of *Apiospora* have been reported to have a cosmopolitan distribution, occurring in terrestrial and marine environments (Klemke et al., 2004), these species are considered uncommon and occasionally understudied. In one study, for example, Kwon et al. (2022) found 242 isolates of nine *Apiospora* species in different bamboo organs in Korean forests. The lifestyle of *Apiospora* specimens is variable, mainly saprobes (Crous et al., 2021; Tian et al., 2021), including coprophiles (Alfatafta et al., 1994), but there are symbiotic (Wang et al., 2017) and endophytic species (Park et al., 2020; Liao et al., 2023), as well as plant (Liao et al., 2022, 2023; Agustí-Brisach et al., 2023) and human pathogens (Paul et al., 2024).

Studying a phytopathogenic alga from blackberry stems (Cortés Martínez et al., 2023), we isolated a fungus with colony morphology and spores similar to *Apiospora* or *Arthrinium* Kunze. Conidia and conidiophores are difficult to distinguish among the species of those genera (Pintos and Alvarado, 2021; Liao et al., 2023). Even *Arthrinium* was previously considered to be the sexual phase of *Apiospora* (Pintos et al., 2019). It was not until the study by Pintos and Alvarado (2021), with sequences from four molecular markers (ITS, 28S, TEF1-α, tub2) and phylogenetic analyses, that it was possible to define both genera as sister lineages.

In Mexico, to our knowledge, there are no reports of *Apiospora* species. The closest taxon is *Arthrinium* sp., which was detected in samples from glacier ice on the volcano Iztaccíhuatl at 5010 m a.s.l. by Calvillo-Medina et al. (2020).

The objective of this study was to identify the fungus that was isolated from the stem of a blackberry plant (*Rubus* 'Tupy') by means of phylogenetic analysis of the sequences of the internal transcribed spacer (ITS) and the translation elongation factor 1-alpha gene (TEF1-α), and determine whether it represents a pathogenic organism of blackberry.

Materials and Methods

Collection site and isolation of fungus

The fungus under study was isolated from the blackberry stems of *Rubus* 'Tupy' affected by orange blotch (Cortés Martínez et al., 2023). The material was collected on April 4, 2019, in the locality of Taretan, Michoacán, Mexico. The climate in the area is humid subtropical with summer rains, annual rainfall of 1560 millimeters and temperatures ranging from 14.4 to 29.7 °C (INAFED, 2020).

Blackberry stem fragments (0.5 cm^2), after three seconds of vortexing (Scientific Industries, G560 SI-0236, Vernon Hills, USA) in 96% alcohol, were rinsed with distilled water, vortexed with 3% chlorine for three seconds (Agrios, 2005), and rinsed three times with sterile distilled water. The material was then placed on potato dextrose agar (PDA, MCD Lab, Mexico) in darkness. The unique isolate obtained of this fungus was purified and labeled as INE47. Samples of the isolate were preserved in 30% glycerin and maintained at 4 °C in the "Colección de organismos benéficos y patógenos del Instituto de Ecología, A.C. (INECOL), Centro Regional del Bajío" in Pátzcuaro, Michoacán, Mexico.

DNA extraction and sequencing

For molecular identification, the total DNA was extracted from the fresh isolate, using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to manufacturer´s instructions. The internal transcribed spacer 1 (ITS1), 5.8S rDNA and ITS2 were amplified by PCR with the primers ITS1F/ITS4 (White et al., 1990; Gardes and Bruns, 1993). Also, the translation elongation factor 1-alpha (TEF1-α) was amplified with primers EF1-688F/EF1-1251R (Alves et al., 2008). In both cases, ITS and TEF1-α, the 25 µl PCR mixture contained 2.5 µl of DNA, 2.5 µl of buffer 5X colorless, 1 µl of 50 mM MgCl2, 1 µl of 10 mM dNTPs, 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 0.2 µl of 5U Taq polymerase (Promega, USA) and 15.8 µl of sterilized water. ITS PCR cycling parameters were as follows: one cycle at 94 °C for 3 min, followed by 30 cycles with a denaturation step at 94 °C for 30 sec, an annealing step at 50 °C for 30 sec, an extension step at 72 °C for 1.15 min, and a final extension at 72 °C for 7 min. The TEF1-α amplification program consisted of one cycle at 95 °C for 5 min, followed by 30 cycles

with a denaturation step at 94 °C for 30 sec, an annealing step at 55 °C for 45 sec, an extension step at 72 °C for 90 sec, and a final extension at 72 °C for 10 min, as described by Phillips et al. (2005). Amplified PCR products were sent to Macrogen (Seoul, Korea) for purification and sequencing reactions. Fungal sequences were deposited in GenBank (2024).

Phylogenetic analyses

The fungal ITS and TEF1-α sequences were assembled using BioEdit v. 7.2.5 (Hall, 1999). A phylogenetic analysis was performed integrating an ITS rDNA matrix (ITS1-5,8S-ITS2 region) and also a second matrix for the TEF1-α marker, with related sequences of the *Apiospora* species referred in Crous et al. (2015), Kwon et al. (2021), Tian et al. (2021), and the most similar published species-sequences deposited in NCBI (2024) (Table 1), including IOM 325295 of *Arthrinium* sp. from the Iztaccíhuatl glacier (Calvillo-Medina et al., 2020) in order to know whether it is related to INE47. Sequences from *Arthrinium* were added and *Nigrospora guangdongensis* L.Y. Tian, a sister taxon of *Apiospora* and *Arthrinium,* was used to root the tree. Sequences in each matrix were aligned using the Mafft program v. 7 (Katoh et al., 2019). The molecular evolution that best fits each data set was determined using jModel test v. 0.1.1 (Posada, 2008). Phylogenetic inference analyses were performed by the Maximum Likelihood (ML) method with 1000 replicates of bootstrap in the program MEGA v. 11.0.10 (Tamura et al., 2021), and Bayesian inference (BI) using the program MrBayes v. 3.2.7a (Ronquist et al., 2012) for 1000000 replicates. The phylogenies from ML and BI analyses were displayed in MEGA v. 11.0.10 (Tamura et al., 2021) or FigTree v. 1.4.3 (Rambaut, 2016), respectively.

Morphological characterization

The mycelial colony was characterized in PDA and in malt-extract agar media (MEA, MCD Lab, Mexico) after two weeks of being maintained at 25 °C in darkness. The colors of its front and rear in the Petri dish were registered using as a reference the color codes of the Methuen handbook (Kornerup and Wanscher, 1983). Semipermanent preparations were made with PVLG (polyvinyl alcohol-glycerol) and 3% KOH to observe the micromorphological structures with a Carl Zeiss optical microscope (Primostar 1, Göttingen, Germany). Size, shape, and color of the spores, conidiophores and conidiogenic cells were recorded in a Leica optical microscope (Serie Galen III, Buffalo, USA). The Zeiss Zen Model 2012 software and an AxioCam ERc 5s camera (Göttingen, Germany) were used to take pictures.

Pathogenicity test

To test the capacity of producing damage in the stems and leaves, fresh mycelia of INE47 were inoculated in detached healthy tissues of blackberry (*Rubus* 'Tupy'). The surface of stems and leaves were disinfested with 70% ethanol for 30 sec, then washed with sterilized water (da Silva et al., 2016). Leaves and stems were wounded with a puncture (1 mm diameter) made with a sterilized dissecting needle (1 mm). Then, 5 mm agar mycelium squares were inoculated on the puncture. In addition, an inoculum square was placed on an area of the tissue without lesion. Controls were inoculated just with an agar square without mycelia in a punctured and an unpunctured area. The test was performed in triplicate and maintained in a humidity chamber (80-100% humidity) for eight days at 25 °C, with a light period of 8 h. Next, if a lesion was observed on tissues, the disease severity was assessed by measuring the lengths of the spots. To confirm whether the lesion was caused for the inoculated organism, small fragments (1-2 mm) of the affected tissues were placed on PDA media. Once the fungus grew, fungal colony and conidia were compared with originally inoculated *Apiospora* to conclude the Koch's postulates.

Results

Molecular identification

Ensambled fungal ITS and TEF1-α sequences from INE47 were uploaded to GenBank (2024) under accession number PQ459871 (ITS) and PQ468997 (TEF1-α). Comparison of the ITS sequence with the BLAST (2024) tool in NCBI shows its high similarity with *Arthrinium intestini* Kajale, Sonawane & Roh. Sharma, whose accepted name is *Apiospora intestini* (Kajale, Sonawane & Roh. Sharma)

Table 1: Sequences and GenBank (2024) accession numbers of *Apiospora* Sacc. species used for phylogenetic analyses. *Nigrospora* Zimm. and *Arthrinium* Kunze sequences were used as outgroup. Samples added in this study are indicated in bold. (-) Information not available.

Species	Strain/voucher	Country	Substrate/Host	ITS accession number	TEF1-α accession number
Apiospora aquatica (Luo, Hyde & Su) Pintos & Alvarado	CC.XQDXG234	China	*Neyraudia reynaudiana* (Kunth) Keng ex Hitchc.	PP407937.1	PP496624.1
Apiospora arundinis (Corda) Pintos & Alvarado	MUCL 14429	Japan	-	AB220280.1	-
Apiospora bambusicola (Tang, Hyde & Kang) Tian & Tibpromma	MFLU 20-0528	Thailand	Bamboo	NR_171983.1	-
Apiospora bambusicola	MFLUCC 20-0144	Thailand	Dead culms of bamboo	MW173030.1	MW183262.1
Apiospora chiangraiense Tian & Tibpromma	MFLU:21-0046	Thailand	Bamboo and maize	MZ542520.1	-
Apiospora chiangraiense	KUNCC 24-17543	China	Poaceae sp.	PP584687.1	PP933194.1
Apiospora intestini Kajale, Sonawane & Roh. Sharma) Pintos & Alvarado	IRAN 4331C	Iran	*Quercus brantii* Lindl.	PP464101.1	PP565758.1
Apiospora intestini	CBS 135835	India	Grasshopper	NR_154698.1	KR011351.1
Apiospora intestini	**INE47**	**Mexico**	**Blackberry**	**PQ459871**	**PQ468997**
Apiospora italica (Pintos & Alvarado) Pintos & Alvarado	AP221017	Italy	*Arundo donax* L.	MK014880.1	MK017956.1
Apiospora jatrophae (Sharma, Kulk. & Shouche) Pintos & Alvarado	CBS:134262	India	*Jatropha podagrica* Hook.	NR 154675.1	-
Apiospora jiangxiensis (Wang & Cai) Pintos & Alvarado	CC.HLG100.4	China	An unidentified woody plan	PP407913.1	PP496602.1
Apiospora lageniformis Kwon & Kim	KUC21686	South Korea	*Phyllostachys nigra* var. *henonis* (Mitford) Stapf ex Rendle	ON764022.1	ON806626.1
Apiospora montagnei Sacc.	AP101020	Spain	*Arundo donax*	ON692409.1	-
Apiospora montagnei	AP301120	Spain	*Arundo micrantha* Lam.	ON692408.1	ON677182.1
Apiospora ovata (Crous) Pintos & Alvarado	CBS 115042	Hong Kong	*Arundinaria hindsii* Munro	NR 121558.1	KF145037.1
Apiospora paraphaeosperma (Senan. & Hyde) Pintos & Alvarado	KUC21688	South Korea	Bamboo	ON764025.1	ON806629.1
Apiospora pseudosinensis (Crous) Pintos & Alvarado	CBS:135459	Netherlands	Bamboo	NR_121559.1	KF145044.1
Apiospora rasikravindrae (S.M. Singh, Yadav, P.N. Singh, Rah. Sharma & S.K. Singh) Pintos & Alvarado	MFLU:23-0237	Thailand	*Ananas comosus* (L.) Merr.	OR438411.1	OR500340.1
Apiospora saccharicola (Stevens) Pintos & Alvarado	CBS:463.83	Netherlands	*Phragmites australis* (Cav.) Trin. ex Steud.	KF144921.1	KF145053.1
Apiospora serenensis (Larrondo & Calvo) Pintos & Alvarado	SFC20220920-B248	South Korea	Sea sand	OP597860.1	-
Apiospora sphaerosperma Pers.) Pintos & P. Alvarado	CBS:114314	Iran	*Hordeum vulgare* L.	KF144904.1	KF145038.1
Arthrinium caricicola Kunze & Schmidt	CBS:145903	Germany	*Carex ericetorum* Pollich	NR 175149.1	
Arthrinium crenatum Pintos, Alvarado & Gardiennet	CBS 146353	France	*Festuca burgundiana* Auquier & Kerguélen	NR_175664.1	MW221917.1
Arthrinium pusillispermum Kwon, Jang & Kim	KCTC 46906	South Korea	Seaweed	NR_177480.1	-
Arthrinium pusillispermum	KUC21321	South Korea	Seaweed	MH498533.2	MN868930.1
Arthrinium sp.	IOM 325295	Mexico	Iztaccíhuatl Glacier	MH890621.1	-
Nigrospora guangdongensis Tian	CFCC 53917	China	-	NR_174814.1	MT024493.1

Pintos & P. Alvarado (culture from holotype: CBS 135835, accession number NR_154698.1), with 99.63% of identity and 96% query coverage, as well as to *Apiospora intestini* (strain IRAN 4331C, accession number PP464101.1) with 99.81%, and 91% of query coverage. The second closest species is *Arthrinium pusillispermum* S.L. Kwon, S. Jang & J.J. Kim (holotype, KCTC 46906m, accession number NR_177480.1), with 99.43% of identity and 92% of query coverage. Whereas the TEF1-α sequence of INE47 is close to *Apiospora intestini* (holotype, CBS 135835, KR011351.1), with 99.44% of identity, this sequence comes from the grasshopper gut, isolated in India, while the second closest sequence, with 98.49% percentage identity, is *Arthrinium intestini* (MZ546406.1), whose host are dead culms of bamboo from Thailand.

In the phylogenetic analyses, a total of 47 sequences (28 ITS and 19 TEF1-α) were incorporated, including the two sequences obtained in this research. The data matrix corresponding to the ITS1+5.8S+ITS2 marker database consisted of 28 sequences, with a 595 bp length. On the other hand, 19 sequences were integrated in the TEF1-α database, with a total alignment length of 552 bp. For the ITS data, the best fitting evolutionary model was TIM3ef+I+G, while for the TEF1-α sequences it was TrNef+G.

Phylogenetic trees based on the TEF1-α sequence showed higher resolution in the *Apiospora intestini* clade than those based on ITS analysis. The concatenated sequence analysis based on ITS and TEF1-α sequences (1147 characters, including gaps) showed a similar topology in Maximum Likelihood and Bayesian analyses, with the latter showing higher resolution (Fig. 1).

Through the multigene analysis of the ITS and TEF1-α, INE47 was identified within the *Apiospora* genus. *Arthrinium* species were located in the phylogenetic analysis between *Apiospora* strains and *Nigrospora guangdongensis* (Fig. 1). INE 47 clustered with *A. intestini* of the holotype (CBS 135835) from India and the strain from IRAN (4331C) with significant statistical support (BI posterior probability=0.99, ML bootstrap=95). *Apiospora arundinis* (Corda) Pintos & P. Alvarado and *A. chiangraiense* X.G. Tian & Tibpromma were the sister clades of INE47.

The glacier sequence (IOM 325295) corresponds to *Apiospora* and is a sister clade of *A. intestini*. Additional molecular markers would clarify the species identity of this sequence.

Taxonomy

Apiospora intestini (Kajale, Sonawane & Roh. Sharma) Pintos & P. Alvarado, Fungal Systematics and Evolution 7: 206. 2021. Fig. 2.

≡ *Arthrinium intestini* Kajale, Sonawane & Roh. Sharma (as ´gutiae´), Persoonia 35: 315. 2015.

TYPE: INDIA. Pune, Western Ghats, Maharashtra, gut of grasshopper, 10.I.2013, *S. Kajale & M. S. Sonawane* (holotype: MCC H1002, culture ex-type MCC 1077 = CBS 135835).

Culture: Mycelium grows fast, covering the entire Petri dish (9 mm) in 3-4 days on PDA and MEA at 25 °C; colony on PDA (Fig. 2A) white, cottony-flat texture, compact, margin irregular, with aerial hyphae forming erect mycelial clusters spreading in some parts of the Petri dish, sporulation scattered, blackish at maturity; reverse side (Fig. 2B) yellowish white to pale yellow (Methuen code: 3A2-3) with spots, concentric circles or olive-brown and greyish-brown rays (Methuen code: 4D3, 4F3 and 5D3); mycelium on MEA (Fig. 2C) beige, loose, scattered over the surface, slightly aerial when forming thin and shorts cords that rise above the surface; pale orange-brown pigments appear as the days go by (Methuen code: 5B2-3); spores forming black spots, aggregates; reverse side (Fig. 2D) yellowish-brown and greyish yellow, with scattered darker brown stains (Methuen code: Bb3-4, 4B3).

Description: Asexual morph: hyphae, thin-walled, smooth, hyaline, septate and branched; conidiophores (Fig. 2E-G) macronematous, hyaline, bunch-shaped, 26-50 × 2 µm, conidia clustered (Fig. 2F, G); conidiogenous cells short cylindrical and hyaline (2 × (1-)2 µm); conidia
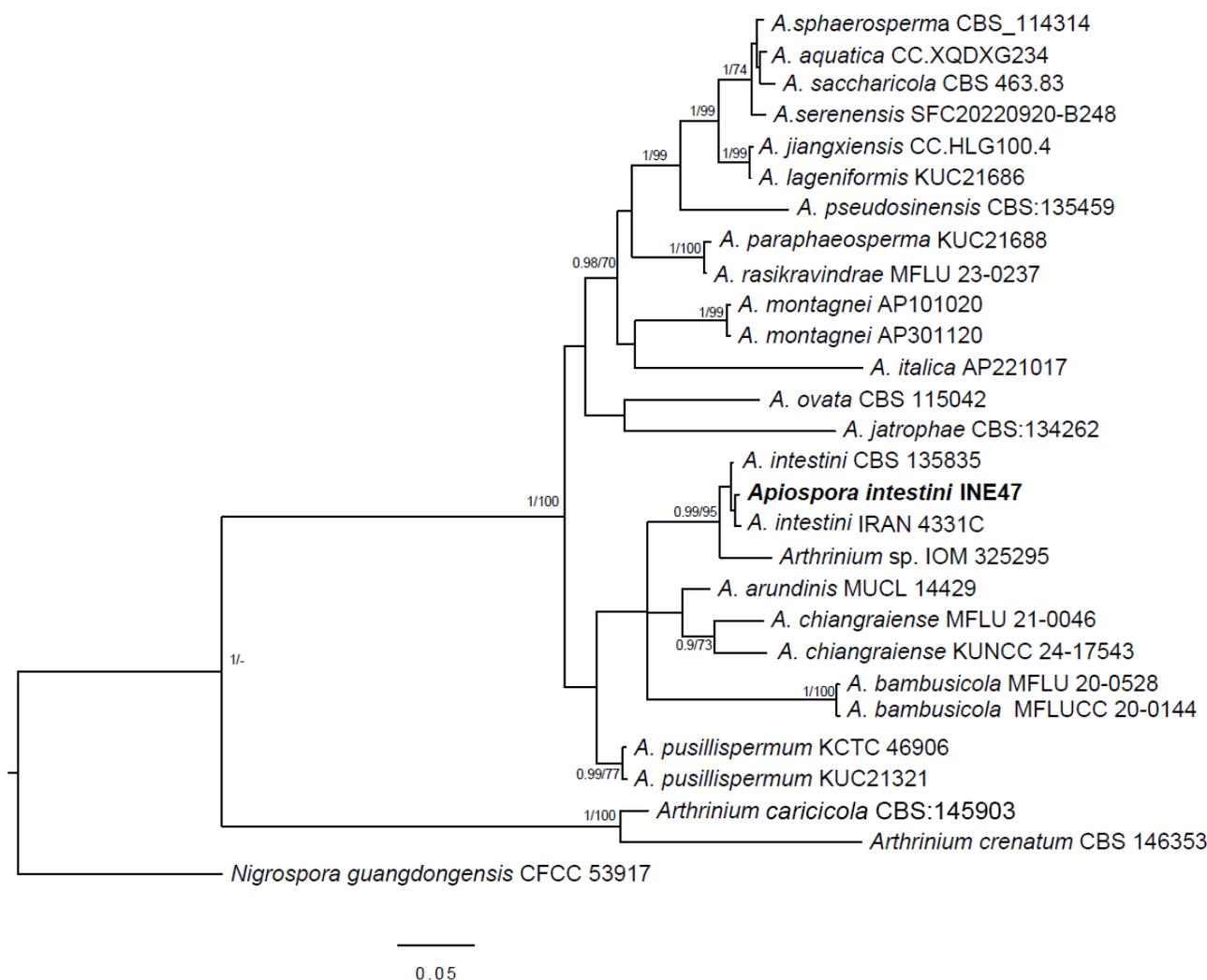
Figure 1: Multigene phylogenetic analysis constructed with Bayesian inference from a concatenated ITS and TEF1-α dataset. *Nigrospora guangdongensis* L.Y. Tian was used to root the tree. Bayesian posterior probability ≥0.95 and bootstrap ≥70 ML are indicated near the nodes. The *Apiospora intestini* (Kajale, Sonawane & Roh. Sharma) Pintos & P. Alvarado sequence from this study is indicated in bold letters.

aseptate, brown, amerospores, globose in surface view (5-7 × 5-6 μm), citriform in side view (6-7 × 4-5 μm) with a scar and pale slit in the middle part along the length of the conidium, thick wall (Fig. 2G, H). Sexual morph: not observed.

Material examined: MEXICO. Michoacán, municipality Taretan, 1170 m, 19°20'00''N, 101°55'00''W, isolated from blackberry stem affected by *Cephaleuros virescens* s.l., 4.IV.2019, *J. Cortes Martinez and E. Garay Serrano INE47* (IEB).
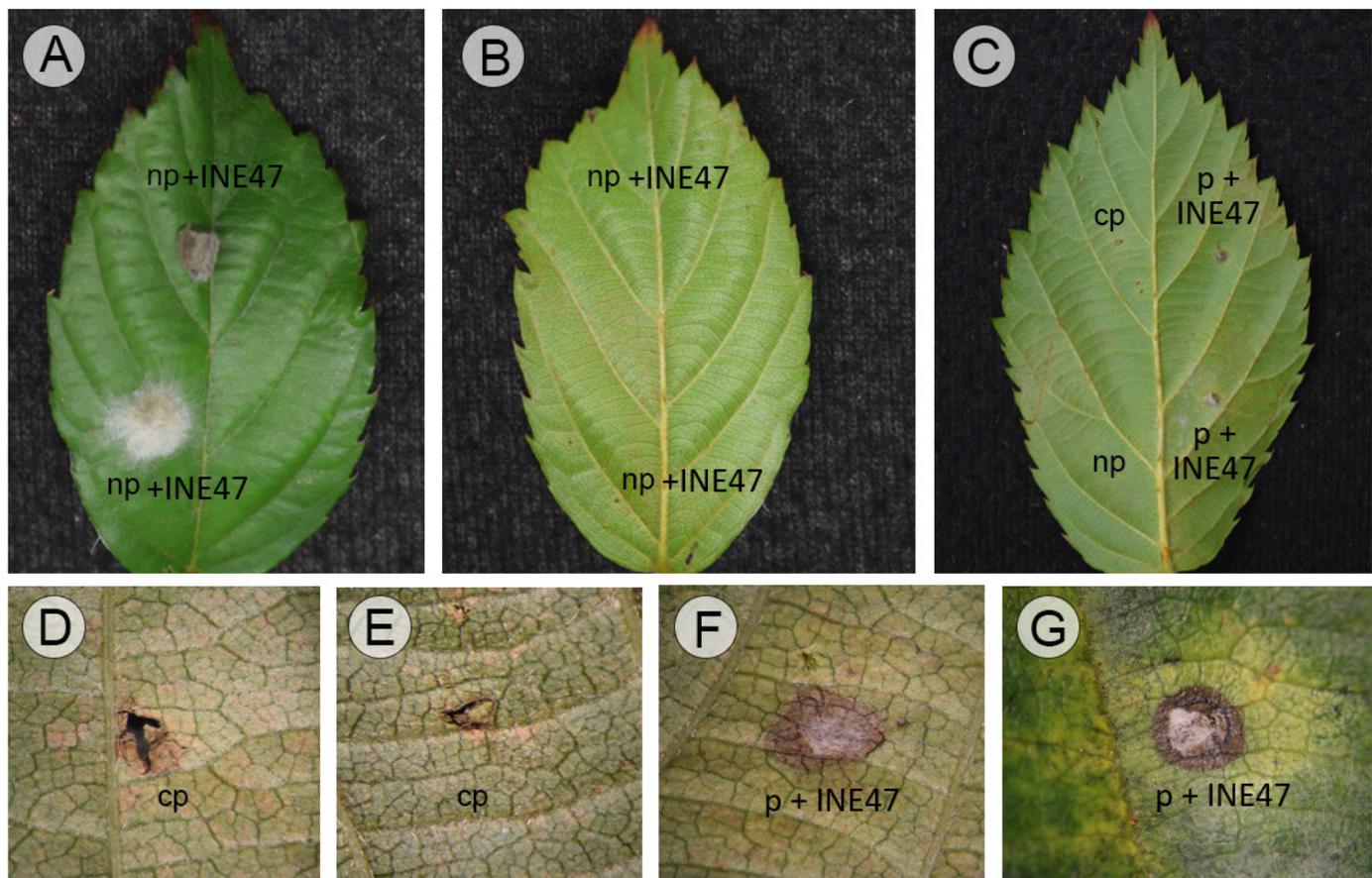
Pathogenicity test

In the experiment of inoculating mycelium in blackberry stems and leaves (Figs. 3, 4), INE47 only induced disease in the punctured tissue, resulting in oval or rounded necrotic spots with a lesion diameter of 2-4 mm on leaves and 2-5 mm on stems after eight days post-inoculation. The same pathogen was re-isolated (100%) from the circular spots of the affected tissues, proving that *A. intestini* is the causative agent of these lesions. However, in the vegetal tissues without puncture, there was no damage or stain of disease induced by INE47.
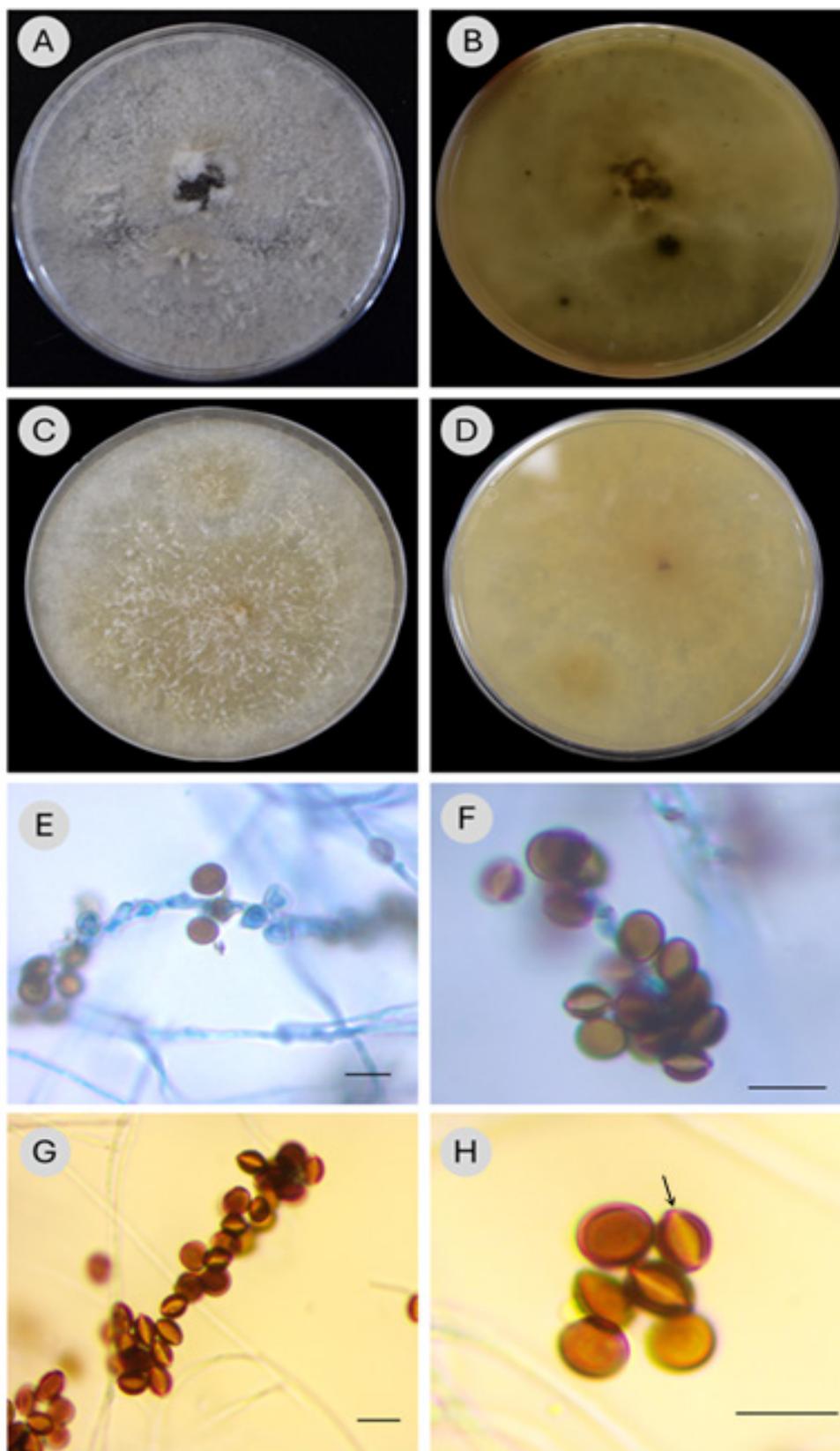
Figure 2: Morphological features of *Apiospora intestini* (Kajale, Sonawane & Roh. Sharma) Pintos & P. Alvarado (INE47). A-B. culture morphology of colony grown in PDA (A, obverse; B, reverse); C, D. in MEA (C, obverse; D, reverse); E, F. conidiophore and brown conidia, stained with trypan blue 0.05%; G, H. conidia brown, globose or citriform in side view with a scar and lighter slit in the middle part of the conidium (arrow). Scale bars=10 µm.
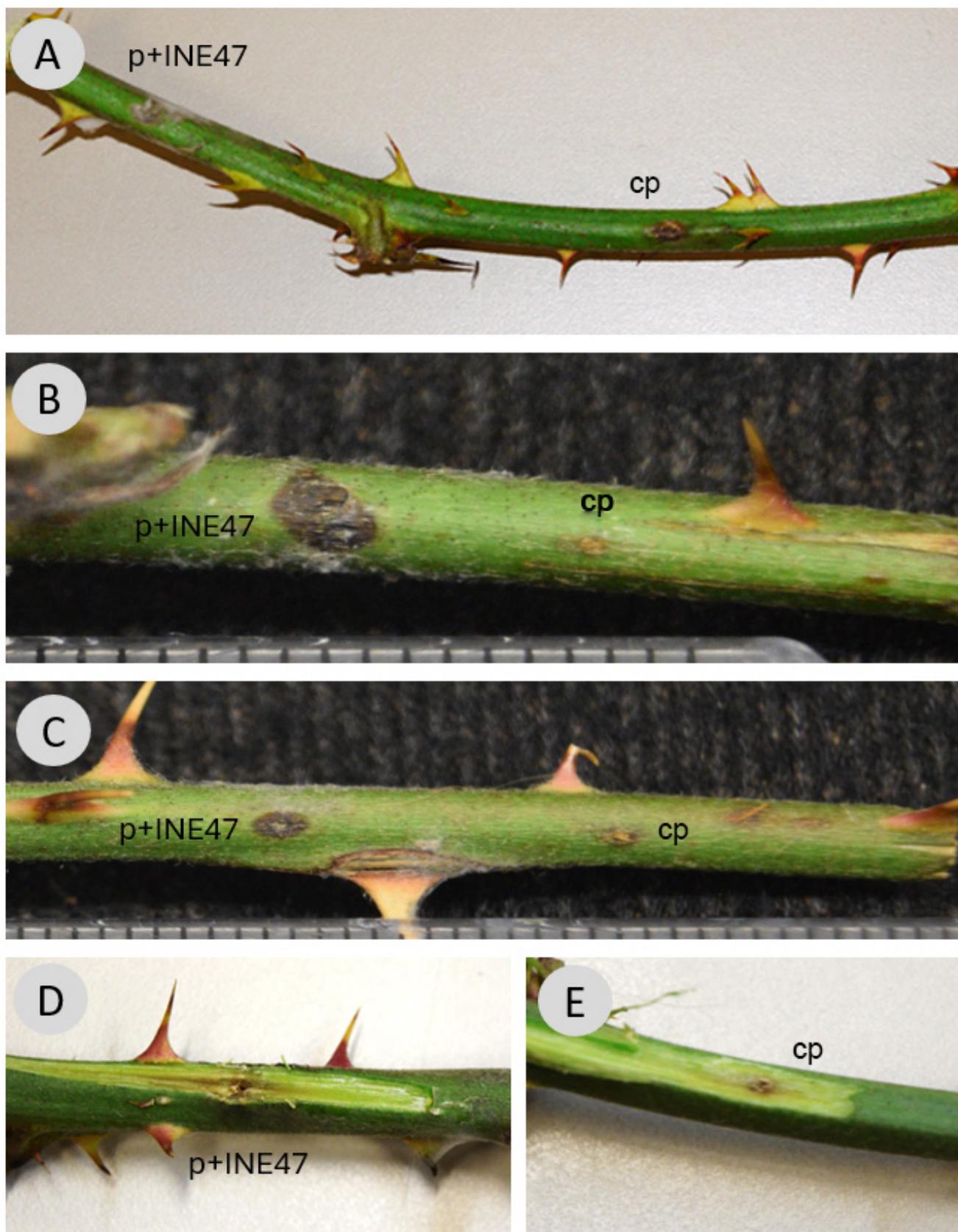
Figure 3: Pathogenicity test with punction on blackberry stems. A-C. INE47 inoculated on punctured area (left side: p+INE47), and control with punctured area without inoculum (right side: cp); D. subepidermal lesion induced by inoculation of INE47; E. control: punctured subepidermal area without inoculum.

Figure 4: Pathogenicity test on blackberry leaves. A-B. INE47 inoculated on non-punctured leaf (np+INE47), showing the adaxial (A) and abaxial side of the same leaf (B), no lesion was induced for the fungus; C. on the left side of the leaf, without injury: control with punction (cp) and, non-punctured (np), while small spots on the right side because of the inoculation of INE47 in a punctured area (p+INE47); D, E. control areas just with punction; F, G. oval and round necrotic lesion caused by INE47 in a punctured area.

Discussion

INE47 detected in the sample of blackberry 'Tupy' was identified as *Apiospora intestini*, as it clustered with the holotype strain of the species (CBS 135835) and the strain (4331C) of Iran, with significant statistical support (BI/ML = 0.99/95). Morphological characteristics confirmed this identity, because of the conidia shape (brown, amerospores, subglobose in side view with a pale equatorial slit), and the disposition of conidia that form spore bunches, conidiophores macronematous with similar sizes (21.5-50 × 2-2.5 µm) in Crous et al. (2015) and 26-50 × 2 µm for INE47. Moreover, the diameter of conidia is almost the same, in INE47 5-6 µm in surface view, in comparison with the type of *A. intestini* (4.5-)5.5(-6) µm (Crous et al., 2015), while in lateral view it is thinner in the type ((2-)4(-6) µm) than in INE47 (4-5 µm).

The closest sister species in the phylogeny, *Apiospora arundinis* (Corda) Pintos & P. Alvarado has spores similar in size: (5-)6-7 µm, in surface view (Crous and Groenewald, 2013), but they are arranged in star-shaped conidiophores rather than in long clusters. However, it is the molecular analyses that mainly support the differentiation of these species (Pintos and Alvarado, 2021) as observed in figure 1, where *A. intestini* is a sister clade of *A. arundinis*.

Apiospora intestini was discovered in the gut of a grasshopper in India in 2015 and described as *Arthrinium gutiae* Kajale, Sonawane, Rohit Sharma (Crous et al., 2015). Subsequently, Pintos and Alvarado (2021), in the separation of *Arthrinium* and *Apiospora* species, recognized it under the current name (*A. intestini*). Since its discovery, few records of this species have been documented, with

different lifestyles: as saprobic in dead culms of bamboo in Thailand (Tian et al., 2021), as pathogen on leaves and stems of *Quercus brantii* Lindl. seedlings, causing very weak symptoms (Bashiri and Abdollahzadeh, 2024), and in *Quercus infectoria* Oliv. (Ghobadi and Jamali, 2025), both in reports from Iran, and the latest record, as endophyte in the medicinal plant *Maytenus emarginata* (Willd.) Ding Hou (Shahim et al., 2024). In this study, the disease severity caused by *A. intestini* on blackberry is limited to a small area. Therefore, at least now, it does not represent an important plant pathogen for this host as it only induces localized necrotic spots in the inoculated areas (Figs. 3, 4). The lesion caused by INE47 is small, and the pathogen is able to establish in the host only through a wound in the stem. Therefore, INE47 could be considered as an opportunistic fungus (Bashiri and Abdollahzadeh, 2024).

There are few reports of *Apiospora* species in Rosaceae: *Apiospora arundinis* in rotten loquat fruit (*Eriobotrya japonica* (Thunb.) Lindl. (Thambugala et al., 2018), and *Apiospora dichotomanthi* (Wang & Cai) Pintos & Alvarado found on *Dichotomanthes tristaniicarpa* Kurz (Wang et al., 2018). Therefore, this report represents, to the best of our knowledge, the first record of *A. intestini* in a Rosaceae species.

The specimen of *Apiospora* from the Iztaccíhuatl glacier (Calvillo-Medina et al., 2020) and INE47 of this study represent the only two records of the genus in Mexico. The present research extends the worldwide *Apiospora intestini* distribution and contributes to the knowledge of Mexican fungal diversity, also broadening the understanding of pathogen lifestyle in a new host.

Author contributions

JCM and EGS obtained vegetative material. JCM performed the laboratory work, designed the tables and figures and compiled the information on the species. EGS performed the molecular work, phylogenetic analyses and drafted the paper. EGS and JCM interpreted the data, and both reviewed and approved the final manuscript.

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Data availability statement

The entire dataset supporting the results of this study was published in SciELO Data and can be accessed at https://doi.org/10.48331/scielodata.DRCCO8

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