New record of *Golovinomyces ambrosiae* on *Solanum azureum* (Solanaceae) in Mexico.

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New record of *Golovinomyces ambrosiae* on *Solanum azureum* (Solanaceae) in Mexico.

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Abstract

We report the powdery mildew *Golovinomyces ambrosiae* for the first time on silverleaf nightshade (*Solanum azureum*), a host plant endemic to northern Sinaloa and southern Sonora, Mexico. No teleomorph was observed, requiring the fungal species to be identified based on morphological characteristics of the anamorph. Phylogenetic analyses of the ITS region and the partial sequence of the β -tubulin gene from four specimens confirmed the identity of the species. To our knowledge, this is the first report of *G. ambrosiae* on *S. azureum* in Mexico and worldwide.

Keywords: Powdery mildew, silverleaf nightshade, endemic host, ITS, TUB2.

Introduction

Powdery mildews (PM) are members of the family Erysiphaceae in the order Erysiphales (Takamatsu 2013a) that infect angiosperms such as agricultural crops and ornamentals (Bushnell 2002) with a worldwide distribution (Ellingham and Culham 2019). Since the teleomorphs are only rarely found in tropical or subtropical regions, the characteristics of the anamorphs may be used for preliminary identifications of PM species. Additionally, substantiated identifications of these phytopathogenic fungi often requires confirmations by molecular techniques (Ellingham and Culham 2019).

The causal agents of PM are obligate parasites (Nicot et al. 2002). The mycelium is typically epiphytic, except in the genera *Leveillula*, *Phyllactinia*, *Pleochaeta*, and in one species of *Cystotheca* in which the mycelium is hemiendophytic. Furthermore, in *Cystotheca*, the pathogen produces superficial hyphae that penetrate the leaves through the stomata and produce mycelia inside, in contrast to species of *Leveillula* where mycelia are abundant

within the leaf of the host plant (Braun et al. 2002). Although the study of PM in Sinaloa, Mexico is in its initial stages, reports of PM diseases include *Podosphaera xanthii* (Castagne) U. Braun and Shishkoff in cucurbits and husk tomatoes (*Physalis philadelphica* Lam.); *Erysiphe quercicola* S. Takam. and U. Braun (= *Pseudoidium anacardii* (F. Noack) U. Braun and R. T. A. Cook) on mango; *Erysiphe diffusa* (Cooke and Peck) U. Braun and S. Takam. on common bean; and *Podosphaera pannosa* (Wallr.) de Bary on roses (Félix-Gastélum et al. 2016). In addition, *Golovinomyces spadiceus* (Berk. and M.A. Curtis) U. Braun has been reported on wild sunflower (Félix-Gastélum et al. 2019). Furthermore, *Phyllactinia* (*Ovulariopsis* cf. *insolita*) has been reported on *Funastrum clausum* (Jacq.) Schltr. and *F. cynanchoides* (Decne.) Schltr. (Bojórquez-Ramos et al. 2014). Recently, *Phyllactinia chubutiana*, was reported on *Vallesia glabra* (Cav.) Link (Félix-Gastélum et al. 2021). In the winter of 2021, signs of PM were observed on silverleaf nightshade (*Solanum azureum* Fernald) for the first time in Mexico. This solanaceous desert shrub is endemic to northern Sinaloa and southern Sonora, Mexico (Fig. 1A) (Knapp et al. 2017; <https://herbanwmex.net/portal/taxa/index.php?taxon=28402&clid=3810>). Although this shrub grows along irrigation canals, railroads, and abandoned lots in urban areas, it has not been reported to compete with agricultural crops in the region.

The initial signs of PM disease on silverleaf nightshade consist of small, powdery white lesions 0.5 – 2.0 mm in diameter (Fig. 1B). As the disease progresses, the lesions cover both the upper (Fig. 1C) and lower leaf surfaces. We observed that structures of the anamorph of the pathogen were copious on infected leaves, while the teleomorph was not observed during the sampling period. To our knowledge, this is the first report of this powdery mildew on *S. azureum* in Mexico.

Materials and methods

Sample collection and light microscopy of asexual structures

Four samples each consisting of 20 symptomatic leaves were collected in the municipality of Ahome, Sinaloa from January 8 through March 10, 2021 ([Table 1](#)). Symptomatic plants were identified at the species level, and a representative sample was deposited at the herbarium of the Universidad Autónoma de Occidente in Los Mochis, Sinaloa, Mexico.

Measurements of somatic and asexual structures were performed in fresh specimens by touching the lesions with a transparent sticky tape and mounting it in a drop of 0.4% cotton blue in lactic acid on a microscope slide. Mountings were observed under a compound microscope (Labomed; Labo America, Inc., USA). Forty fungal structures of the anamorph from four sampling sites were measured (n=160). The presence or absence of fibrosin bodies in fresh conidia was determined according to previously described procedures ([Kable et al. 1963](#)). To examine conidial germination, conidia were transferred from naturally infected leaves to sterilized microscope slides using a smooth-bristled brush. Subsequently, conidia (n=80) were incubated for 24 – 48 h at 100% relative humidity and $23 \pm 2^{\circ}\text{C}$. The length of the germ tube and its width at the midpoint were measured. Light micrographs of conidiophores and conidia were obtained with a microscope (Leica DM6000 CS, LAS AF version 2.5.0.6735), while the images of hyphal appressoria and germinated conidia were acquired using a compound microscope (Axio Imager M2; Carl Zeiss, Göttingen, Germany).

DNA extraction, PCR, and sequencing

For molecular identification, a small amount (~50 mg) of somatic and reproductive structures of four specimens (*Sa-1–Sa-4*) were transferred from infected leaves to 1.5-mL Eppendorf tubes using a fine paintbrush. Genomic DNA extraction was performed using 500 μL of DNAzol® (Thermo Fisher Scientific; Cincinnati, OH, USA) according to the manufacturer's instructions. The extracted DNA was resuspended in 30 μL of nuclease-free water, and then

one μL of a ten-fold dilution was used as a template for the first round of nested PCR with the primer set ITS1/ITS4 (White et al. 1990). One μL of this first reaction was used for amplification with primers ITS4 and the Erysiphaceae-specific primer PM5 (Takamatsu and Kano 2001). Additionally, a PCR reaction was conducted using the TubF1/TubR1 primer set to amplify ~ 450 bp of the partial β -tubulin (TUB2) gene sequence (Qiu et al. 2020).

The PCR mixes contained 2.5 μL of buffer, 0.75 μL of 50 $\mu\text{mol/L}$ MgCl_2 , 1.0 μL of each primer (10 $\mu\text{mol/L}$), 1 μL of 10 mmol/L dNTP mix, 0.1 μL (1 U) of Taq DNA polymerase (Invitrogen, USA), 1.0 μL of template DNA, and nuclease-free water for a final volume of 25 μL . PCR amplifications were performed in an Apollo ATC-201 PCR cycler (Nyx Technik; San Diego, CA, USA), using the following program conditions: an initial denaturing step at 94°C for 5 min; 35 cycles consisting of 30 s at 94°C followed by 30 s at 58°C (ITS) or 52°C (TUB2) for annealing and 30 s at 72°C for extension; and a final extension cycle of 5 min at 72°C . The PCR products were sent for purification and sequencing to Macrogen Inc (Seoul, Korea).

Phylogenetic analysis

Sequences were edited in BioEdit v 7.0.5.3 (Hall 1999) and aligned with reference sequences of the *G. ambrosiae* complex and related species (Qiu et al. 2020), using the MUSCLE algorithm (Edgar 2004) implemented in MEGA X (Kumar et al. 2018). The best-fitting partitioning scheme was selected using unlinked branch lengths, greedy algorithm, and the Akaike Information Criterion (AIC) in PartitionFinder v 1.1.1 (Lanfear et al. 2012). Finally, the phylogenetic analysis was conducted with Maximum Likelihood (ML) in RAXML v 7.2.8 (Stamatakis 2006), using the GTRGAMMAI model for each partition identified by Partition Finder and 1000 rapid bootstraps.

Results

Morphological characteristics

Since the teleomorph of the fungus was not found during the sampling period, all morphological studies were performed on the anamorph. We observed ectophytic-amphigynous mycelium of PM on infected plants. Hyphal width was (3.7–) 5.0 – 7.5 (–10.0) μm (5.6 μm on average), with solitary nipple-shaped hyphal appressoria (Fig. 1D). Conidiophores (*Euoidium*-type) emerged from the superficial mycelium, measured (12.5–) 87.5 – 100.0 (–162.5) μm in length $\times$ (5.0–) 10.0 – 12.5 (–15.0) μm in width (107.1 $\times$ 10.5 μm on average) with straight foot cell of (25.0–) 25.0 – 37.5 (–90.0) μm in length $\times$ (3.2–) 5.0–7.5 (–15.0) μm in width (38.5 $\times$ 6.3 μm on average). Conidia were ellipsoid–ovoid and cylindrical, without any fibrosin bodies (Fig. 1F), (25.0–) 32.5–37.5 (–45.0) μm in length $\times$ (10.0–) 15.0–17.5 (–22.5) μm in width (32.9 $\times$ 16.0 μm on average), with an average length/ratio of 2.0. Germ tubes were simple, emerging on shoulders, rarely at the end of the conidia (Fig. 1G), and measured (15.0–) 25.0–27.5 (–42.5) μm in length $\times$ (2.5–) 2.5–2.7 (–5.0) μm in width (26.4 $\times$ 2.9 μm on average).

Phylogenetic analysis

Sequences of the ITS region and the TUB2 gene were compared with sequences in the NCBI database using the BLAST and MegaBLAST algorithms. The comparison of ITS sequences revealed a 99.78% identity with *G. ambrosiae*, *G. spadiceus* and *G. latisporus* (GenBank accession numbers MT355557, KX821733 and MK452603, respectively). A 99.17% – 100% identity with *G. ambrosiae* and *G. latisporus* (GenBank accession numbers MK389490 and MK452495, respectively) was recorded using the TUB2 sequences.

The final combined alignment (ITS + TUB2) consisted of 648 bp. PartitionFinder suggested the same partition for the ITS region and all codon positions (first, second and third) of the

TUB2 gene (lnL= -1325.61). The inferred phylogram from the combined alignment showed the grouping of four specimens associated with silverleaf nightshade (*S. azureum*) to *G. ambrosiae*, in a well-supported clade (99% bootstrap; Figure 2). Specimen sequences were deposited in GenBank with the following accession numbers: MW934451-MW934453 for ITS, and MW929327-MW929330 for TUB2 (Table 1).

Discussion

Our results show that the morphological features of the anamorph of the PM associated with *Solanum azureum* coincide with those reported as *Golovinomyces spadiceus* from Sinaloa, Mexico on wild sunflower (Félix-Gastélum et al. 2019), and can be distinguished from *G. latisporus* on *Helianthus* spp. [previously referred to as *G. ambrosiae* (Braun and Cook 2012)], based on morphological characteristic. Phylogenetic analyses of the ITS region and the TUB2 gene revealed a correspondence with *G. ambrosiae*, including *G. spadiceus* as synonym, as recently reassessed and re-circumscribed on the basis of multi-locus phylogenetic analysis, in which *G. ambrosiae* and *G. spadiceus* proved to be conspecific (Qiu et al. 2020). Individual and combined gene analysis strongly grouped both species names in the same clade, which was renamed as *G. ambrosiae* (containing *G. spadiceus*). Widely distributed throughout Australia, Asia, Europe and North America, this plurivorous species occurs on *Ambrosia* spp., as well as numerous species of various genera belonging to the Asteraceae, Fabaceae, Solanaceae and Verbenaceae families (Qiu et al. 2020). The incidence of *G. ambrosiae* on *S. azureum*, a plant endemic to northern Sinaloa and southern Sonora, Mexico (Knapp et al. 2017; <https://herbanwmex.net/portal/taxa/index.php?taxon=28402&clid=3810>), is relevant since this fungus is referred to as plurivorous, even though it has not yet been reported associated with any member of the Solanaceae in Mexico.

Several *Golovinomyces* spp. have been reported on members of Solanaceae (Cunnington et al. 2005, Braun and Cook 2012). Braun et al. (2019) listed members of Solanaceae among the hosts of *G. spadiceus* (now *G. ambrosiae*), which goes back to Takamatsu et al. (2013b), who included a sequence retrieved from a North American powdery mildew sample on *Solanum carolinianum* in Lineage III. To our knowledge this is the first report of *G. ambrosiae* on a member of Solanaceae in Mexico and the first record of this species on *Solanum azureum*. Future research projects should be directed at determining the incidence of *G. ambrosiae* on other solanaceous plants in Sinaloa, including agricultural crops important to this region.

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252

253 **Table 1.** Data for *Golovinomyces ambrosiae* associated with *Solanum azureum*.

Sample	Locality	Collection date (in 2021)	Coordinates	GenBank Accession no.	
				ITS	TUB2
<i>Sa-1</i>	Cahuinahua	January 8	26° 31' 15" N 109° 12' 44" O	-	MW929327
<i>Sa-2</i>	Cahuinahua	January 8	26° 02' 44" N 109° 13' 08" O	MW934451	MW929328
<i>Sa-3</i>	Canal Tabeojeca	March 10	26° 01' 55" N 109° 13' 58" O	MW934452	MW929329
<i>Sa-4</i>	Canal Tabeojeca	March 10	26° 01' 21" N 109° 13' 44" O	MW934453	MW929330

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Figure captions

Figure 1. Signs of powdery mildew *Golovinomyces ambrosiae* specimen *Sa-4* on leaves of *Solanum azureum*, and morphological characteristics of the fungus. A: Flowers and healthy leaves of the host. B: Initial signs consisting of small, whitish circular lesions on the adaxial surface of the leaf. C: Leaf covered with white powdery mildew. D: Light micrograph depicting single nipple-shaped appressoria on the hyphae (black arrow). E: Conidiophore with attached conidium. F: Conidium with *Euoidium*-type germination (black arrow). G: Ovoid- to cylindrical-shaped conidia. Scale bars: D, F and G = 20 µm, E=15 µm.

Figure 2. Maximum likelihood tree based on the combined ITS + TUB2 sequence datasets from the *Golovinomyces ambrosiae* complex, *G. magnicellulatus* and *G. circumfusus*. *Neoerysiphe galeopsidis* was used as an outgroup. Sequences of the specimens infecting *Solanum azureum* are shown in bold. Numbers above the branches indicate bootstrap nodal support. The scale bar indicates the number of nucleotide substitutions per site.

235x265mm (300 x 300 DPI)

395x155mm (300 x 300 DPI)