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Peronospora variabilis is associated with downy mildew of *Chenopodium berlandieri* in Mexico

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Abstract: *Peronospora variabilis* was observed to be consistently associated with downy mildew of pitseed goosefoot (*Chenopodium berlandieri*). Morphological characteristics of the conidiophores, conidia and oospores of the oomycete corresponded to those of *P. variabilis.* The morphological identification was complemented by a phylogenetic analysis of the ITS region. To our knowledge, this is the first report of *P. variabilis* on *C. berlandieri* in Mexico.

Key words: *Peronospora variabilis*, downy mildew, *Chenopodium berlandieri*, pitseed goosefoot, chenopod weed.

Introduction

Pitseed goosefoot (*Chenopodium berlandieri* Moq.) is a weedy annual herbaceous plant in the family Amaranthaceae. This species is widespread in North America, where its native habitat extends from Alaska and northern Canada south to Michoacán, Mexico, and includes every U.S. state except Hawaii (Wilson 1990). This plant is used as a spinach-like vegetable in Mexico. In Sinaloa, the plant competes with crops such as dry bean and corn during the fall-winter growing season, although it is also found along roadsides, mainly in alluvial soils. No diseases have been reported in this weed in Mexico; however, in recent years, symptoms and signs of a disease resembling downy mildew have been observed in plants competing with dry bean in February in northern Sinaloa, Mexico. The initial symptoms consist of minute chlorotic lesions (0.2-0.3 mm in diameter) on the adaxial surface of leaves. As these lesions increase in size, they become irregular in shape and pinkish to reddish in color (Fig. 1A), with limited sporulation on the corresponding abaxial leaf surface. In advanced stages of the disease, the whole leaf including the petiole becomes reddish (Fig. 1B) and defoliation may occur. Preliminary observations in the Laboratory of Plant Pathology at the Universidad Autonoma de Occidente indicated the presence of conidia of a *Peronospora* species (Webster and Weber 2007) on the abaxial part of the leaves, corresponding to the reddish area on top of the leaves. The disease was observed during February, during which daily leaf wetness periods varied from 13-16 h and temperatures ranged from 8-26°C.

Downy mildew, caused by *P. variabilis* Gäum. (formerly known as *Peronospora farinosa* f. sp. *chenopodii* Byford) is an endemic disease on *C. quinoa* Willd. in Peru, where it causes yield losses of 20-25% (Alandia et al. 1979). The disease has also been observed on the weeds *C. album* L., *C. murale* L. and *C. ambrosioides* L. (Aragón and Gutiérrez 1992). In addition, quinoa downy mildew disease caused by *P. variabilis* has been reported in Denmark (Danielsen et al. 2002), the USA (Testen et al. 2012; Nolen et al. 2022), South Korea (Choi et al. 2014), Turkey (Kara et al. 2020) and Italy (Beccari et al. 2021). Although *P. variabilis* has been reported on weedy chenopods in South America (Aragón and Gutiérrez 1992), the identity of the *Peronospora* species associated with pigweed goosefoot (*C. berlandieri)* in Mexico is so far unknown. This study therefore aimed to identify at the species level the Oomycete associated with downy mildew on this plant.

Materials and Methods

Sample collection and light microscopy of reproductive structures

Five samples each consisting of 30 symptomatic leaves were collected from five dry bean fields at maturity stage from February 12 to 23, 2022 in the municipality of El Fuerte, Sinaloa, Mexico (Table 1). Plant specimens were collected in the field, pressed flat between newspapers and dried. The host plant was identified at the species level and deposited at the herbarium of the Universidad Autonoma de Occidente in Los Mochis, Sinaloa.

The shape and measurements of conidiophores, conidia and oospores were determined in fresh specimens by touching the grey lesions on the abaxial part of symptomatic leaves with a transparent sticky tape and mounting it in a drop of 60% lactic acid on a microscope slide. For the oospores, fragments of infected tissue were crushed in a drop of the same fluid on a microscope slide, and then a cover slip was placed over the sample. Preparations of the asexual structures (n=250) and the oospores (n=133) were observed under a compound microscope (Labomed Microscope; Labo America, Inc., USA). Micrographs of conidiophores, conidia and oospores were taken with a microscope (Axio Imager A2; Carl Zeiss, White Plains, NY, USA) to characterize their morphology.

Data on measurements are described as maxima and minima in parentheses, and the mean plus and minus the standard deviation of a number of measurements is provided in

parentheses. The means are presented in italics in the center of the data (between two values), as previously described (Choi et al. 2010).

DNA extraction, PCR, and sequencing

For genomic DNA extraction, approximately 100 mg of symptomatic leaf tissue was ground in a 2-mL Eppendorf tube containing 1 mL of CTAB buffer (2% CTAB, 100 mM Tris HCl pH 8.0, 20 mM EDTA, and 1.4 M NaCl) (Doyle 1991). The extracted DNA was diluted in 100 µL of nuclease-free water and used as a template to amplify a ribosomal region with the primer set DC6/LR-0 (Cooke et al. 2000).

The PCR mixes contained 2.5 µL of buffer, 0.75 µL of 50 µmol/L $MgCl_2$, 1.0 µL of each primer (10 µmol/L), 1.0 µL of 10 mmol/L dNTP mix, 0.1 µL (0.5 U) of Taq DNA polymerase (Invitrogen; USA), 0.5 µL of DNA, and nuclease-free water for a final volume of 25 µL. The PCR amplifications were performed in a C1000 Touch PCR thermal cycler (Bio Rad; Hercules, CA, USA) with the following program conditions: 5 min at 95°C for initial denaturing; 35 cycles consisting of 1 min at 95°C followed by 40 s at 58°C for annealing and 2 min at 72°C for extension; and a final extension step of 5 min at 72°C. The PCR products were sent for purification and sequencing to Macrogen, Inc. (Seoul, South Korea).

Phylogenetic analysis

The sequences were edited in BioEdit version 7.0.5.3. (Hall 1999) and compared in the NCBI GenBank database using the BLAST-N software and the MegaBLAST algorithm. MEGA 11 (Tamura et al. 2021) was used for alignment and phylogenetic reconstruction. An alignment was constructed using the MUSCLE aligner (Edgar 2004) and reference sequences from the genus *Peronospora* (Choi et al. 2008; Choi et al. 2010; Lee et al. 2020). The sequence of *Peronospora lamii* D104 was used as an outgroup. Multiple alignment was subjected to a DNA substitution model analysis to select the model that best fits the data, according to the Akaike Information Criteria (AIC). Phylogenetic reconstruction was performed using Maximum Likelihood method (ML) and the General Time Reversible model with a gamma distribution of rates across sites and invariant sites (GTR+G+I). Tree topology support was

assessed by 1000 bootstrap replicates. The obtained phylogram was edited with FigTree 1.4.0 (Rambaut 2010).

Results

Morphological characteristics

All morphological studies were performed with fresh *C. berlandieri* leaves infected with the *Peronospora variabilis*. conidiophores that emerged through the stomata were sub-dichotomously branched mostly slightly curved (75–) 141 *218* 385 (–395.0) µm (n=250) long (n=250) (Fig. 2A); basal end of the conidiophore (5–) 8 *11* 16 (–18), with branchlets mostly in pairs (Fig. 2B); trunk straight to slightly curved, from the base to the first branch (25–) 83 *127* 242 (–300) µm (n=250); branchlets occurred at an obtuse angle with a tapered tip variable in size (5-) 12 *16.0* 21 (27) (Fig. 2B). Conidia were light brown and ovoid to ellipsoid in shape with a short conical pedicel (12–) 16 *27* 34 (–37) µm long (n=250), (12–) 15 *21* 29 (–32) µm wide (Fig. 2C), and a length ratio (1.0–) 1.17 -*1.28* –1.53 (–1.7). Oospores that were orange in color and (15-) 17 *18* 16 (- 23) µm (n=133) in diameter were present in the leaf tissue in oogonia with a sub-globose to irregular shape and (17–) 20 *23* 25 (–27) µm in diameter (n=133) (Fig. 2D). Oospores were observed in all specimens except in *Chb-2* (Table 1).

Phylogenetic analysis

The comparison of ITS sequences of the five specimens from Sinaloa, Mexico revealed a 99.4-100% identity with those of *P. farinosa* (GenBank accession numbers AY211018 and AF528556), and a 99.8% identity with *P. variabilis* (GenBank accession number MT666070). All sequences were deposited in the GenBank database (Table 1). The inferred phylogram displays the grouping of all specimens with the reference sequences of *P. variabilis* isolates HMAS57036 (EF614959) and SMK18830 (EF614964) (65% bootstrap; Figure 3).

Discussion

Five samples of *C. berlandieri* from Sinaloa, Mexico with downy mildew symptoms indicated a constant association with an Oomycete from the genus *Peronospora* (Webster and Weber, 2007). Despite some differences in the dimensions of the conidiophores, conidia and oospores, the morphological characteristics of our specimens correspond to those of *Peronospora variabilis* (formerly known as *P. farinosa*) infecting *C. quinoa* in Argentina, Bolivia, Denmark, Ecuador and Peru (Choi et al. 2010), the USA (Testen et al. 2012), South Korea (Choi et al. 2014) and Turkey (Kara et al. 2020).

Although the dimensions of the reproductive structures of our specimens from Sinaloa differed from those of *P. variabilis* from *C. album* (Choi et al. 2008) and *C. quinoa* (Choi et al. 2010), the morphology of our five specimens from *C. berlandieri* were similar to those from *C. album* in Argentina, Bolivia, Denmark, Ecuador, and Peru (Choi et al. 2010). These differences in the dimensions of the reproductive structures may be due to the host plant and the climatic conditions in which the disease occurs during the winter in Sinaloa, Mexico.

In this study, we complemented the morphological identification of our specimens with molecular techniques. The phylogenetic analysis based on the ITS region confirmed the identity of our specimens (*Chb*-1 to *Chb*-5) as *P. variabilis*. Furthermore, *C. berlandieri* is allied to *C. album*, the type host of this species.

Several *Peronospora* species are known as implicated as causal agents of downy mildew on *Chenopodium* (Gäumann 1919). However, since no morphological differences were found between these species, it was concluded that *P. farinosa* was the only species involved in the disease (Yerkes and Shaw 1959). Later, morphological and molecular studies have supported the separation of five groups at the host species level, suggesting that *P. variabilis*, *P. boni-henrici* Gäum., *P. chenopodii* Schltdl. and *P. chenopodii-polyspermi* Gäum. are causal agents of downy mildew on *C. album*, *C. bonus-henricus* L., *C. hybridum* L. and *C. polyspermum* L., respectively (Choi et al. 2008). The conidiophore branching and conidial shape similar to those of *P. variabilis*; furthermore, the inferred phylogram from the ITS sequences grouped our specimens with *P. variabilis* in an independent branch of the above-mentioned species. In Sinaloa, *C. berlandieri* is considered a weed, and its nutritional attributes remain unknown. Other weedy species of *Chenopodium* occurring in Mexico include *C. ambrosioides* and *C. murale* (Calderón de Rzedowski and Rzedowski 2004), which have been reported to be

infected by *P. variabilis* in Peru (Aragón and Gutierrez 1992). The incidence of downy mildew in these plant species in Mexico remains unknown.

Although a high degree of physiological specialization has been found in the family Peronosporaceae (Crute 1981), *P. variabilis* is reported to be pathogenic to *C. album* and *C. quinoa* (Choi 2010). To the best of our knowledge, this is the first report of *P. variabilis* on *C. berlandieri* in Mexico. Future research should focus on determining the incidence of the disease, as well as identifying the species of *Peronospora* associated with the disease on *C. ambrosioides* and *C. murale* in Mexico. This will conduct to the implementation of control measurements of the disease in this *Chenopodium* species.

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Author Statements

Competing Interests The authors declare there are no competing interests

Data Availability Data available within the article

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Table 1. Data on *Peronospora variabilis* associated with *Chenopodium berlandieri*

Sample	**Locality**	**Altitude**	**Collection date (2022)**	**Coordinates**	**GenBank Accession no.**
Chb-1	Charay	26 m.a.s.l.	February 12	26° 00’ 24” N 108° 48’ 12” W	ON314874
Chb-2	Charay	26 m.a.s.l.	February 12	26° 00’ 24” N 108° 48’ 59” W	ON314875
Chb-3	Charay	26 m.a.s.l.	February 23	25° 58’ 05” N 108° 47’ 16” W	ON314876
Chb-4	Charay	26 m.a.s.l.	February 23	25° 59’ 05” N 108° 47’ 23” W	ON314877
Chb-5	Charay	26 m.a.s.l.	February 23	25° 59’ 45” N 108° 47’ 43” W	ON314878

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254

255 **Figure legends**

256 Fig. 1. Symptoms of downy mildew *Peronospora variabilis* on leaves of *Chenopodium*
257 *berlandieri*. (A) Minute chlorotic lesions on the adaxial part of the leaves, which increase in
258 size and become irregular in shape and pinkish in color. (B) Leaf blades and petioles invaded
259 by the pathogen in the lower part of the plant becoming pinkish-red in color.

260 Fig. 2. Morphological characteristics of *Peronospora variabilis*. (A) Dichotomously
261 branched conidiophore. (B) Ultimate branchlets appear curved and tapered at their ends. (C)
262 Ellipsoidal conidia with a pedicel (back arrow). (D) Smooth oogonium with a globose
263 oospore.

264 Fig. 3. Maximum likelihood tree based on the ITS region of *Peronospora*. The sequence of
265 *Peronospora lamii* was used as an outgroup. Sequences of the specimens that infect
266 *Chenopodium berlandieri* are shown in green boldface. Bootstrap values greater than 50%
267 are shown above the branches. The scale bar indicates the number of nucleotide substitutions
268 per site.

Fig. 1. Symptoms of downy mildew Peronospora variabilis on leaves of Chenopodium berlandieri. (A) Minute chlorotic lesions on the adaxial part of the leaves, which increase in size and become irregular in shape and pinkish in color. (B) Leaf blades and petioles invaded by the pathogen in the lower part of the plant becoming pinkish-red in color.

255x171mm (150 x 150 DPI)

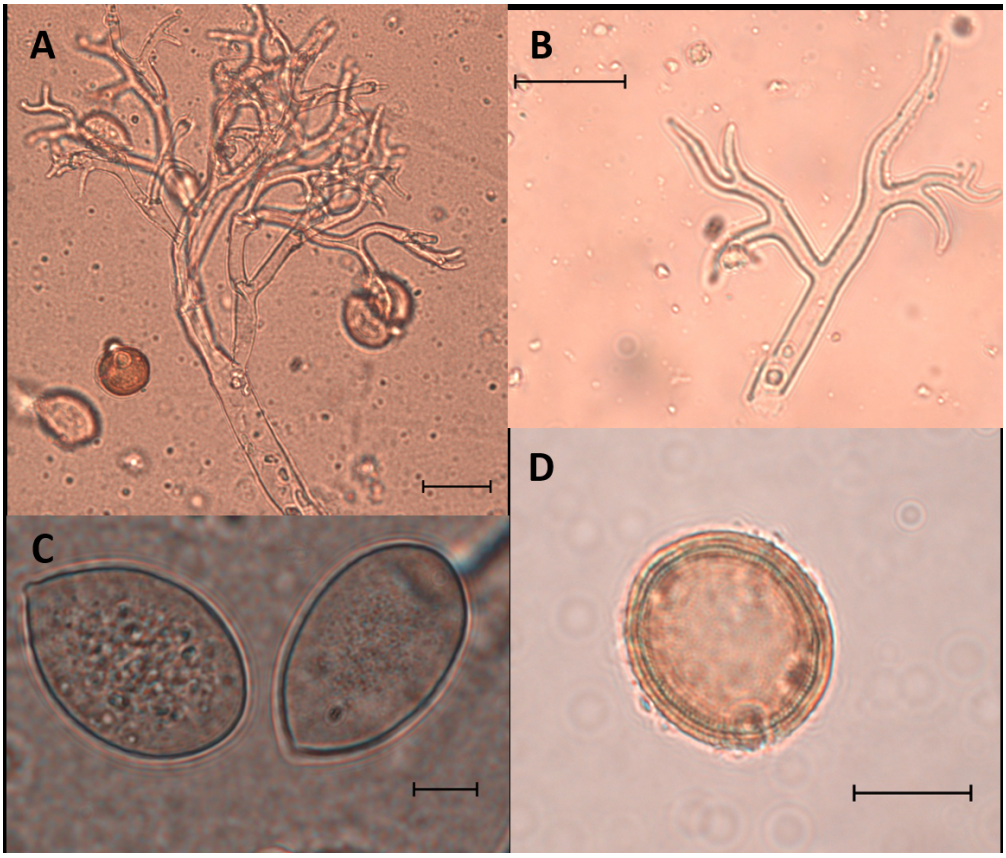

Fig. 2. Morphological characteristics of Peronospora variabilis. (A) Dichotomously branched conidiophore. (B) Ultimate branchlets appear curved and tapered at their ends. (C) Ellipsoidal conidia with a pedicel (back arrow). (D) Smooth oogonium with a globose oospore.

195x166mm (150 x 150 DPI)

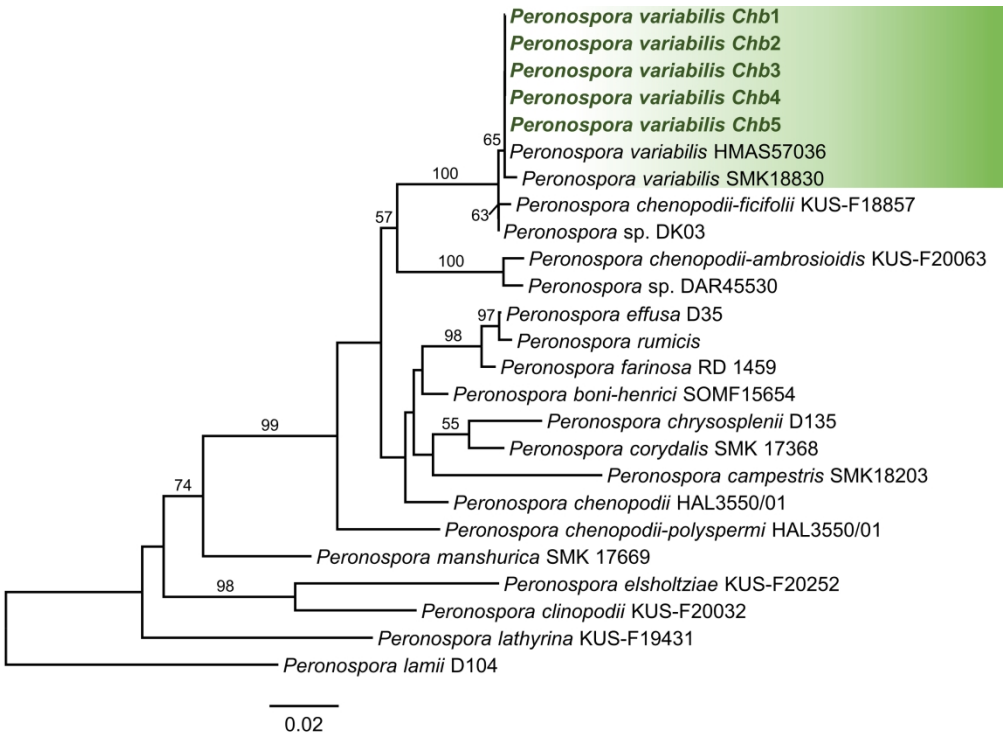

Fig. 3. Maximum likelihood tree based on the ITS region of Peronospora. The sequence of Peronospora lamii was used as an outgroup. Sequences of the specimens that infect Chenopodium berlandieri are shown in green boldface. Bootstrap values greater than 50% are shown above the branches. The scale bar indicates the number of nucleotide substitutions per site

263x193mm (300 x 300 DPI)