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# *Phyllachora* species infecting maize and other grass species in the Americas represents a complex of closely related species

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## Abstract

The genus *Phyllachora* contains numerous obligate fungal parasites that produce raised, melanized structures called stromata on their plant hosts referred to as tar spot. Members of this genus are known to infect many grass species but generally do not cause significant damage or defoliation, with the exception of *P. maydis* which has emerged as an important pathogen of maize throughout the Americas, but the origin of this pathogen remains unknown. To date, species designations for *Phyllachora* have been based on host associations and morphology, and most species are assumed to be host specific. We assessed the sequence diversity of 186 single stroma isolates collected from 16 hosts representing 15 countries. Samples included both herbarium and contemporary strains that covered a temporal range from 1905 to 2019. These 186 isolates

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were grouped into five distinct species with strong bootstrap support. We found three closely related, but genetically distinct groups of *Phyllachora* are capable of infecting maize in the United States, we refer to these as the *P. maydis* species complex. Based on herbarium specimens, we hypothesize that these three groups in the *P. maydis* species complex originated from Central America, Mexico, and the Caribbean. Although two of these groups were only found on maize, the third and largest group contained contemporary strains found on maize and other grass hosts, as well as herbarium specimens from maize and other grasses that include 10 species of *Phyllachora*. The herbarium specimens were previously identified based on morphology and host association. This work represents the first attempt at molecular characterization of *Phyllachora* species infecting grass hosts and indicates some *Phyllachora* species can infect a broad range of host species and there may be significant synonymy in the *Phyllachora* genus.

## 1 | INTRODUCTION

Phyllachorales is a monophyletic order of biotrophic fungi comprised of approximately 1,226 recognized species (Maharachchikumbura et al., 2016; Mardones et al., 2017), but global estimates of species within this order approach 160,000 (Cannon, 1997). The Phyllachorales largely contain plant parasitic fungi and are commonly associated with monocotyledonous plants across a range of habitats. These fungi are often referred to as "tar spot" fungi due to the production of stromata on plant hosts that resemble black flecks of tar (Figure 1) (Mardones et al., 2017).

Tar spot of maize (Figure 1a–c), caused by the fungus *Phyllachora maydis*, emerged in the United States in 2015, with the disease expanding each year since the initial report and continuing to have a significant economic impact on maize across many production regions in the United States (Kleczewski et al., 2020; Valle-Torres et al., 2020). Since first identified in North America in 2015, *P. maydis* has spread rapidly throughout the United States and Canada (Kleczewski & Bowman, 2020; Kleczewski et al., 2020), and resulted in yield losses exceeding \$US 658 million in 2018 (Mueller et al., 2020). Although tar spot symptoms caused by members of the genus *Phyllachora* have been commonly observed on a number of grasses (Figure 1d–f) and shrub species throughout North, Central, and South America, historically the fungus has rarely been known to cause significant plant damage. However, tar spot has been occasionally reported to cause severe damage to maize in Mexico, Central America, and several Caribbean Islands (Valle-Torres et al., 2020).

The origin of *P. maydis* within the United States is not currently known, although the presence of two distinct epicenters of maize tar spot in the Midwest and Southeast indicates at least two separate emergence events. While tar spot is a new disease on maize in

the United States and Canada, it has been present in Mexico, several Caribbean islands including Puerto Rico, Cuba, and the Dominican Republic as well as Central American Countries, such as Guatemala, Honduras, Nicaragua, and Costa Rica for the last century but only caused limited damage. In addition, tar spot signs and symptoms caused by *Phyllachora* species are common on several native and weedy grass species in North America (Figure 1d–f) (Orton, 1944). The monographic work by Orton (1944) was completed solely by morphological identification and host affinity. Given our understanding of phenotypic plasticity of many fungi and the ability of biotrophic pathogens to infect multiple hosts (Morris & Moury, 2019), it is possible that cryptic species or species complexes may be present.

Species definitions within the Phyllachorales have historically been based largely on morphological characteristics and assumption of high host specificity due to their presumed biotrophic nature. However, there are examples in the genus where this assumption of host specificity does not hold true (Cannon, 1991, 1997). Furthermore, species designations based on host specificity are highly dependent on accurate identification of the host species, which may be difficult or impossible in some instances. For example, *P. graminis* (Pers.) Fuckel is considered a "dustbin" species where many specimens of isolates infecting grasses are deposited with the host not often identified to species (Parbery, 1967). Furthermore, factors such as nutrients available to the fungus, temperature, light quality, light cycles, substrate type, host, and epigenetic factors may also result in alterations in fungal morphology that may result in inaccurate species designations (Francisco et al., 2019; Money, 2013; Slepecky & Starmer, 2009; Stockinger et al., 2009). Thus, our current understanding of the genetic diversity, host range, and species delimitation within the genus *Phyllachora* is relatively limited and requires reevaluation.

FIGURE 1 Signs and symptoms of *Phyllachora* spp. on grasses. *Phyllachora maydis* on maize at severe levels (a); with ascospores being extruded from stroma (b) and showing characteristic tapering ends of mature stromata (c). *Phyllachora* spp. on *Elymus* in Michigan (d), Fall Ryegrass in Illinois, and an unidentified grass in Indiana (f). Photo credit N. Kleczewski

The recent emergence of *P. maydis* in the United States and Canada may also be associated with the ability of the fungus to better persist and spread than previously thought. Once established, the fungus can survive at least one winter at subzero temperatures on corn residue as ascospores within stromata, which are believed to be the main inoculum source the following season (Groves et al., 2020; Kleczewski et al., 2019). Under periods of moderate temperatures and wet weather, it is believed that ascospores are dispersed by wind and rain splash where they land on the foliage, stalks, and husks of corn. After spore germination and infection of the host, the fungus remains dormant for at least 2 weeks after which stromata, and associated spermatia and ascospores, are produced. Data from Central America indicated a relatively steep dispersal curve of *P. maydis* ascospores from a source (Hock et al., 1995). However, the rapid spread of this fungus throughout the Midwest, coupled with observations of "top down" infestations in fields with no history of disease and observations of infestations of isolated plots located 1,200 m from potential inoculum sources, indicate that the pathogen can travel much further across local/regional topographies than estimated previously (Kleczewski et al., 2020).

Based on this information, the emergence of *P. maydis* on corn in the United States and Canada could have been the result of many factors including the introduction of the fungus on infected plant material, natural northern dispersal through wind, establishment in the United States favored by climate change, changes in hybrid genetics, a host jump from a grass species, or a combination of any of these four. This study represents the first attempt to extract and sequence DNA from *Phyllachora* stroma on fresh and herbarium-infected grass specimens. The goal of this study is to understand the genetic diversity of *Phyllachora* species causing tar spot disease in contemporary corn production regions in the United States, and compare this to historical specimens of *Phyllachora* species from herbarium samples of maize and other grasses from Mexico, Central, and South America, the Caribbean, and Europe, as well as contemporary and herbarium species of *Phyllachora* species associated with grass hosts in the United States. This represents the first attempt at genetic characterization of *Phyllachora maydis*, *Phyllachora graminis*, and other grass infecting *Phyllachora* species. The objectives of this study are to: (1) determine if a single species of *Phyllachora* is responsible for tar spot disease of maize throughout its range in the Americas over the last century or if distinct genetic groups are responsible for these symptoms; and (2) determine if *Phyllachora* species infecting native and weedy grasses in close proximity to maize production fields are the same species as those infecting maize. Understanding the phylogenetic diversity and the potential host and geographic range of *Phyllachora* species associated with maize and other grasses in the Americas will also help to infer the potential evolutionary origins and speciation patterns in this genus.

## 2 | MATERIAL AND METHODS

### 2.1 | Sample collection

Samples of maize and wild grasses with characteristic stromata of *Phyllachora* spp. (Figure 1a–f) were collected from across North America and Mexico in 2018 and 2019 (Table 1). Field specimens of infested maize and other grasses were collected by numerous individuals from the agricultural community as described in Kleczewski et al. (2020). Samples were pressed, dried at room temperature, and stored at 20°C in manila envelopes until processed. Herbarium

specimens were obtained from the U.S. National Fungus Collection (BPI, Beltsville, Maryland) and the University of Illinois Herbarium (Urbana, Illinois), which included specimens on maize and other grasses from additional hosts, countries, and years (Table 1). A total of 186 samples from 16 hosts and 15 countries, collected from 1905 to 2019, were included in the analyses.

## 2.2 | DNA extraction, PCR amplification, and sequencing of stroma from leaf tissue

The DNA of individual stroma not surrounded by a necrotic halo were extracted using the X-Tract-N-AMP kit following manufacturer protocols (Sigma). The complete internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2) with primers ITS1f and ITS4 (White et al., 1990). Stroma without necrotic halos was selected to reduce the potential for contamination by saprophytic fungi that may be present on necrotic tissue within these lesions.

The ITS gene region was amplified from DNA extracted from each stroma using the primer pair ITS1f and ITS4 (Bruns & Gardes, 1993; White et al., 1990) with 35 cycles of the following: 95°C 5 min, 94°C 30s, 52°C 30s, 72°C 1 min, followed by 72°C for 8 min, and a final hold at 4°C in a Thermo Fisher SimpliAmp thermocycler (Thermo Fisher Scientific, Waltham, WA). Individual PCR products from corresponding DNA extractions were loaded into 2% agarose gels and separated via electrophoresis for 40 min at 110V. All gels contained a *P. maydis*-positive control, a *Fusarium graminearum*-positive control, and a negative buffer control for quality assurance. Bands on gels were visualized using an Axygen gel imaging station (Axygen, Inc., Union City, CA). Stroma of *Phyllachora* spp. can be colonized by or associated with several other fungal species (Hock et al., 1992, 1995; McCoy et al., 2019). Consequently, samples returning a single band between 300 and 500 bp were considered free of additional fungal contaminants and used in subsequent analyses.

DNA from samples returning a single ITS band were subject to amplification of the large ribosomal subunit (LSU) region using the primer pair LR0R and LR5 (Dayarathne et al., 2017) using the aforementioned thermocycler conditions. All PCR products were purified using QIAquick PCR kits (Quiagen, Inc., Hilden, Germany), and the ITS and LSU amplicons for all samples were sequenced in the forward and reverse directions at the University of Illinois Core DNA Sequencing Facility (Urbana, Illinois).

## 2.3 | Sequence alignment, phylogenetic analysis, and molecular identification

Sequences generated from this study were combined with sequences obtained from GenBank. *Exserohilum turcicum* and *Cocoicola californica* were selected as the outgroups. Sequence data were aligned and concatenated using MAFFT v.7 (www.mafft.cbrc.jp/alignment/server/) using the G-INS-I model and manually inspected. The best = fit partitioning schemes were determined using PartitionFinder (Lanfear et al.,

2017) and used to build the phylogenies. Both single gene and concatenated gene sets were analyzed using a maximum likelihood (ML) analysis using RaxML and Bayesian inference with MrBayes. The ML phylogenies were generated by RaxML (Stamatakis, 2014) under GTR model with gamma distributed rate heterogeneity with 1000 bootstrap replicates. For the Bayesian inference, we used MrBayes v. 3.2.6 (Ronquist et al., 2012) using the general time reversible (GTR) model selected for the entire unpartitioned alignment, with the likelihood parameters setting (lset) number of substitution types (nst) = 6, with a proportion of sites invariable and the rest drawn from the gamma distribution (rate = invgamma). Four independent analyses, each starting from a random tree, were run under the same conditions for the combined gene alignment. Three hot and one cold chains of Markov Chain Monte Carlo iterations were performed. Analyses were run with 1,000,000 generations with sampling every 100 generations. The first 250,000 generations were discarded as the chains were converging (burnin period). Resulting trees were visualized with iTOL (Interactive Tree of Life) v.6 (https://itol.embl.de/) or MEGA. Sequences generated in this study were deposited in GenBank (Table 1).

## 3 | RESULTS

### 3.1 | DNA extraction and PCR amplification from herbarium and contemporary samples

A total of 186 samples from 12 states in the United States (*n* = 130), 4 states in Mexico (*n* = 13), 3 Central American countries (*n* = 13), 4 South American countries (*n* = 6), 4 Caribbean Islands (*n* = 16), Germany (*n* = 3), India (*n* = 3), and the Philippines (*n* = 2) were sequenced and analyzed as noted above. There were varying levels of success for the amplification of each genetic locus among the samples. This was particularly the case for many of the herbarium samples, some of which were more than 100 years old. The ITS region was the most successfully amplified and sequenced, with 168 sequences generated. Whereas 91 sequences were generated for the LSU locus (Table 1).

### 3.2 | Phylogenetic diversity of *Phyllachora* isolates infecting maize and grasses

Based on both ITS + LSU (Figure 2) and ITS (Figure S1) phylogenies, we observed five genetically distinct groups that represent individual species of *Phyllachora* with strong bootstrap and posterior probability support (>70%). The results suggest that tar spot on maize in the United States is caused by three closely related species of *Phyllachora* (Figure 2). In all, four species were found on maize but only *Phyllachora* sp. 1, *Phyllachora* sp. 2, and *Phyllachora* sp. 3 were recovered from contemporary maize in the United States, while *Phyllachora* sp. 4 was recovered from herbarium samples collected in Guatemala and Venezuela (Table 1).

Samples of *Phyllachora* sp. 3 represent the broadest geographic and host range and was also the most frequently recovered species

**TABLE 1** The sample ID, genetic cluster, geographic and host origin, year collected, and source of the 186 *Phyllachora* specimens used in this study

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ITS | LSU |
| BPI893226_2 | 1 | *Phyllachora maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342800 | |
| BPI893227_2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342801 | |
| BPI893229_2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342802 | |
| BPI893230_2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342803 | |
| BPI893231_2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342804 | |
| BPI893232_2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2015 | Field collection | OL342805 | |
| C18001-2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342781 | |
| C18001-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342782 | |
| C18003-1 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342783 | |
| C18003-2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | | OL314402 |
| C18003-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342784 | |
| C18009-1 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342785 | |
| C18009-2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342786 | |
| C18011-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342787 | OL314403 |
| C18024-1 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342788 | |
| C18024-2 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342789 | |
| C18024-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342790 | |
| C18161-1 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342791 | OL314404 |
| C18161-2 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342792 | OL314405 |
| C18161-3 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | | OL314406 |
| C18162-1 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342793 | OL314407 |
| C18162-3 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342794 | OL314408 |
| C18164-1 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342795 | |
| C18164-2 | 1 | *P. maydis* | Ohio | USA | *Zea mays* | 2018 | Field collection | OL342796 | OL314409 |
| C18164-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2018 | Field collection | OL342797 | |
| C19043-1 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2019 | Field collection | OL342798 | |
| C19043-3 | 1 | *P. maydis* | Indiana | USA | *Zea mays* | 2019 | Field collection | OL342799 | |
| BPI638548_1 | 2 | *P. maydis* | Cundinamarca | Colombia | *Zea mays* | 1940 | USDA Herbarium | OL342824 | |
| BPI638554_1 | 2 | *P. maydis* | Añasco | Puerto Rico | *Zea mays* | 1917 | USDA Herbarium | OL342825 | |
| BPI638578_1 | 2 | *P. maydis* | Vega Baja | Puerto Rico | *Zea mays* | 1916 | USDA Herbarium | OL342826 | |

(Continues)

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions ITS | GenBank accessions LSU |
|---|---|---|---|---|---|---|---|---|---|
| BPI910562_1 | 2 | *P. maydis* | Michigan | USA | *Zea mays* | 2017 | USDA Herbarium | OL342827 | |
| C18026-3 | 2 | *P. maydis* | Puebla | Mexico | *Zea mays* | 2018 | Field collection | OL342806 | |
| C18030-1 | 2 | *P. maydis* | Guerrero | Mexico | *Zea mays* | 2018 | Field collection | OL342807 | OL314410 |
| C18030-2 | 2 | *P. maydis* | Guerrero | Mexico | *Zea mays* | 2018 | Field collection | OL342808 | OL314411 |
| C18030-3 | 2 | *P. maydis* | Guerrero | Mexico | *Zea mays* | 2018 | Field collection | OL342809 | OL314412 |
| C18031-1 | 2 | *P. maydis* | Veracruz | Mexico | *Zea mays* | 2018 | Field collection | OL342810 | OL314413 |
| C18031-2 | 2 | *P. maydis* | Veracruz | Mexico | *Zea mays* | 2018 | Field collection | OL342811 | OL314414 |
| C18031-3 | 2 | *P. maydis* | Veracruz | Mexico | *Zea mays* | 2018 | Field collection | OL342812 | OL314415 |
| C18033-2 | 2 | *P. maydis* | Oaxaca | Mexico | *Zea mays* | 2018 | Field collection | OL342813 | OL314416 |
| C18033-3 | 2 | *P. maydis* | Oaxaca | Mexico | *Zea mays* | 2018 | Field collection | OL342814 | OL314417 |
| C18038-3 | 2 | *P. maydis* | Guerrero | Mexico | *Zea mays* | 2018 | Field collection | OL342815 | |
| C18040-1 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2018 | Field collection | OL342816 | |
| C18040-2 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2018 | Field collection | OL342817 | |
| C18040-3 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2018 | Field collection | OL342818 | OL314418 |
| C18069-1 | 2 | *P. maydis* | Illinois | USA | *Zea mays* | 2018 | Field collection | OL342819 | OL314419 |
| C18069-2 | 2 | *P. maydis* | Illinois | USA | *Zea mays* | 2018 | Field collection | OL342820 | OL314420 |
| C18069-3 | 2 | *P. maydis* | Illinois | USA | *Zea mays* | 2018 | Field collection | OL342821 | |
| C19001-1 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2019 | Field collection | OL342822 | |
| C19001-2 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2019 | Field collection | OL342823 | |
| C19001-3 | 2 | *P. maydis* | Florida | USA | *Zea mays* | 2019 | Field collection | | |
| 92794-1 | 3 | *P. chaetochloae* | Santiago | Dominican Republic | *Setaria* sp. | 1931 | UIUC Herbarium | OL342860 | |
| 92794-2 | 3 | *P. chaetochloae* | Santiago | Dominican Republic | *Setaria* sp. | 1931 | UIUC Herbarium | OL342861 | |
| 92794-3 | 3 | *P. chaetochloae* | Santiago | Dominican Republic | *Setaria* sp. | 1931 | UIUC Herbarium | OL342862 | |
| 92812-1 | 3 | *P. diplocarpa* | California | USA | *Distichilis spicata* | 1942 | UIUC Herbarium | OL342863 | |
| 92821-1 | 3 | *P. epicampsis* | Arizona | USA | *Muhlenbergia emersleyi* | 1948 | UIUC Herbarium | OL342864 | |
| 92821-2 | 3 | *P. epicampsis* | Arizona | USA | *Muhlenbergia emersleyi* | 1948 | UIUC Herbarium | OL342865 | |
| 92821-3 | 3 | *P. epicampsis* | Arizona | USA | *Muhlenbergia emersleyi* | 1948 | UIUC Herbarium | OL342866 | |
| 92825-1 | 3 | *P. euphorbiaceae* | Mumbai | India | *Euphorbia* sp. | 1932 | UIUC Herbarium | OL342867 | |
| 92825-2 | 3 | *P. euphorbiaceae* | Mumbai | India | *Euphorbia* sp. | 1932 | UIUC Herbarium | OL342868 | |

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions ITS | LSU |
|---|---|---|---|---|---|---|---|---|---|
| 92825-3 | 3 | P. euphorbiaceae | Mumbai | India | Euphorbia sp. | 1932 | UIUC Herbarium | OL342869 | |
| 92845-3 | 3 | P. graminis | Mittelfranken | Germany | Agropyron repens | 1946 | UIUC Herbarium | OL342870 | |
| 92922-3 | 3 | P. heraclei | Hessen | Germany | Heracleum spondylium | 1977 | UIUC Herbarium | OL342871 | |
| 92925-2 | 3 | P. junci | Holstein | Germany | Juncus effusus | 1946 | UIUC Herbarium | OL342872 | |
| 92938-1 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | UIUC Herbarium | OL342873 | |
| 92938-2 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | UIUC Herbarium | OL342874 | |
| 92938-3 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | UIUC Herbarium | OL342875 | |
| 92940-2 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | UIUC Herbarium | OL342876 | |
| 92940-3 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | UIUC Herbarium | OL342877 | |
| 93013-1 | 3 | P. rottboelliae | Luzon | Philippines | Rottboellia | 1931 | UIUC Herbarium | OL342878 | |
| 93013-2 | 3 | P. rottboelliae | Luzon | Philippines | Rottboellia | 1931 | UIUC Herbarium | OL342879 | |
| 93064-1 | 3 | P. sylvatica | California | USA | Festuca idahoensis | 1941 | UIUC Herbarium | OL342880 | |
| 93064-2 | 3 | P. sylvatica | California | USA | Festuca idahoensis | 1941 | UIUC Herbarium | OL342881 | |
| 93126-2 | 3 | P. vulgata | Arizona | USA | Muhlenbergia glauca | 1948 | UIUC Herbarium | OL342882 | |
| 93126-3 | 3 | P. vulgata | Arizona | USA | Muhlenbergia glauca | 1948 | UIUC Herbarium | OL342883 | |
| BPI638546_1 | 3 | P. maydis | Maracas Valley | Trinidad and Tobago | Zea mays | 1945 | USDA Herbarium | OL342884 | |
| BPI638553_1 | 3 | P. maydis | | Mexico | Zea mays | 1904 | USDA Herbarium | OL342885 | |
| BPI638556_1 | 3 | P. maydis | Valle del Cauca | Colombia | Zea mays | 1929 | USDA Herbarium | OL342886 | |
| BPI638558_1 | 3 | P. maydis | Antigua | Guatemala | Zea mays | 1905 | USDA Herbarium | OL342887 | |
| BPI638559_1 | 3 | P. maydis | Matagalpa | Nicaragua | Zea mays | 1956 | USDA Herbarium | OL342888 | |
| BPI638561_1 | 3 | P. maydis | Veracruz | Mexico | Zea mays | 1932 | USDA Herbarium | OL342889 | |
| BPI638564_1 | 3 | P. maydis | | Mexico | Zea mays | 1977 | USDA Herbarium | OL342890 | |
| BPI638567_1 | 3 | P. maydis | Havana | Cuba | Zea mays | 1918 | USDA Herbarium | OL342891 | |
| BPI638568_1 | 3 | P. maydis | Alajuela | Costa Rica | Zea mays | 1947 | USDA Herbarium | OL342892 | |
| BPI638570_1 | 3 | P. maydis | Vega Baja | Puerto Rico | Zea mays | 1916 | USDA Herbarium | OL342893 | |
| BPI638571_1 | 3 | P. maydis | Turrialba | Costa Rica | Zea mays | 1949 | USDA Herbarium | OL342894 | |
| BPI638572_1 | 3 | P. maydis | Chimaltenanco | Guatemala | Zea mays | 1940 | USDA Herbarium | OL342895 | |
| BPI638574_1 | 3 | P. maydis | Arecibo | Puerto Rico | Zea mays | 1917 | USDA Herbarium | OL342896 | |
| BPI638575_1 | 3 | P. maydis | Chimaltenanco | Guatemala | Zea mays | 1942 | USDA Herbarium | OL342897 | |
| BPI638577_1 | 3 | P. maydis | Nor Yungas | Bolivia | Zea mays | 1943 | USDA Herbarium | OL342898 | |

(Continues)

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ITS | LSU |
| BPI638579_1 | 3 | P. maydis | | Guatemala | Zea mays | 1941 | USDA Herbarium | OL342899 | |
| BPI638580_1 | 3 | P. maydis | Santander | Colombia | Zea mays | 1936 | USDA Herbarium | OL342900 | |
| BPI638581_1 | 3 | P. maydis | Vega Baja | Puerto Rico | Zea mays | 1916 | USDA Herbarium | OL342901 | |
| BPI638582_1 | 3 | P. maydis | Guatemala | Guatemala | Zea mays | 1905 | USDA Herbarium | OL342902 | |
| BPI638584_1 | 3 | P. maydis | Antigua | Guatemala | Zea mays | 1905 | USDA Herbarium | OL342903 | |
| BPI638585_1 | 3 | P. maydis | | Guatemala | Zea mays | 1906 | USDA Herbarium | OL342904 | |
| BPI638586_1 | 3 | P. maydis | Lima | Peru | Zea mays | 1929 | USDA Herbarium | OL342905 | |
| BPI638587_1 | 3 | P. maydis | La Vega | Dominican Republic | Zea mays | 1930 | USDA Herbarium | OL342906 | |
| BPI638588_1 | 3 | P. maydis | | Guatemala | Zea mays | 1906 | USDA Herbarium | OL342907 | |
| BPI893226_1 | 3 | P. maydis | Indiana | USA | Zea mays | 2015 | USDA Herbarium | OL342908 | |
| BPI893228_1 | 3 | P. maydis | Indiana | USA | Zea mays | 2015 | USDA Herbarium | OL342909 | |
| BPI893230_1 | 3 | P. maydis | Indiana | USA | Zea mays | 2015 | USDA Herbarium | OL342910 | |
| BPI893231_1 | 3 | P. maydis | Indiana | USA | Zea mays | 2015 | USDA Herbarium | OL342911 | |
| BPI893232_1 | 3 | P. maydis | Indiana | USA | Zea mays | 2015 | USDA Herbarium | OL342912 | |
| BPI893233_1 | 3 | P. maydis | Illinois | USA | Zea mays | 2015 | USDA Herbarium | OL342913 | OL314452 |
| BPI893234_1 | 3 | P. maydis | Illinois | USA | Zea mays | 2015 | USDA Herbarium | OL342914 | OL314453 |
| BPI910560_1 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2017 | USDA Herbarium | OL342915 | |
| BPI910561_1 | 3 | P. maydis | Iowa | USA | Zea mays | 2016 | USDA Herbarium | OL342916 | |
| C18046-1 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | OL342829 | OL314422 |
| C18046-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314421 |
| C18046-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314423 |
| C18047-1 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | OL342830 | OL314424 |
| C18047-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314425 |
| C18047-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314426 |
| C18049-1 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314427 |
| C18049-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | | OL314428 |
| C18050-2 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | OL342831 | |
| C18075-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2018 | Field collection | OL342832 | OL314429 |
| C18119-2 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | | OL314430 |
| C18119-3 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | | OL314431 |

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | ITS | LSU |
| C18136-1 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | | OL314432 |
| C18148-1 | 3 | P. maydis | Iowa | USA | Zea mays | 2018 | Field collection | OL342833 | OL314433 |
| C18148-2 | 3 | P. maydis | Iowa | USA | Zea mays | 2018 | Field collection | OL342834 | OL314434 |
| C18148-3 | 3 | P. maydis | Iowa | USA | Zea mays | 2018 | Field collection | OL342835 | OL314435 |
| C18149-1 | 3 | P. maydis | Iowa | USA | Zea mays | 2018 | Field collection | OL342836 | OL314436 |
| C18149-2 | 3 | P. maydis | Iowa | USA | Zea mays | 2018 | Field collection | OL342837 | OL314437 |
| C18153-1 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | OL342838 | OL314438 |
| C18153-2 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2018 | Field collection | | OL314439 |
| C19007-1 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342839 | OL314440 |
| C19007-2 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342840 | OL314461 |
| C19007-3 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342841 | OL314441 |
| C19008-1 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342842 | OL314442 |
| C19008-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342843 | OL314459 |
| C19008-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | | OL314443 |
| C19012-2 | 3 | P. maydis | Minnesota | USA | Zea mays | 2019 | Field collection | OL342844 | OL314444 |
| C19012-3 | 3 | P. maydis | Minnesota | USA | Zea mays | 2019 | Field collection | OL342845 | OL314445 |
| C19022-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342846 | OL314460 |
| C19022-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342847 | OL314446 |
| C19025-1 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342848 | |
| C19025-2 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342849 | |
| C19025-3 | 3 | P. maydis | Illinois | USA | Zea mays | 2019 | Field collection | OL342850 | |
| C19040-1 | 3 | P. maydis | Michigan | USA | Zea mays | 2019 | Field collection | OL342851 | OL314447 |
| C19040-2 | 3 | P. maydis | Michigan | USA | Zea mays | 2019 | Field collection | OL342852 | OL314456 |
| C19040-3 | 3 | P. maydis | Michigan | USA | Zea mays | 2019 | Field collection | OL342853 | OL314448 |
| C19043-2 | 3 | P. maydis | Indiana | USA | Zea mays | 2019 | Field collection | OL342854 | |
| C19072-1 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2019 | Field collection | OL342855 | OL314449 |
| C19072-2 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2019 | Field collection | | OL314450 |
| C19072-3 | 3 | P. maydis | Wisconsin | USA | Zea mays | 2019 | Field collection | OL342856 | OL314451 |
| C19106-1 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342857 | OL314457 |
| C19106-2 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342858 | OL314458 |
| C19106-3 | 3 | P. maydis | Iowa | USA | Zea mays | 2019 | Field collection | OL342859 | OL314462 |

(Continues)

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | ITS | LSU |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | GenBank accessions | |
| BPI638565 | 4 | *P. maydis* | | Venezuela | *Zea mays* | 1957 | USDA Herbarium | OL342920 | |
| BPI638576 | 4 | *P. maydis* | Mazatenango | Guatemala | *Zea mays* | 1906 | USDA Herbarium | OL342921 | |
| BPI638583 | 4 | *P. maydis* | | Guatemala | *Zea mays* | 1907 | USDA Herbarium | OL342922 | |
| NC19004-1 | 4 | *Phyllachora* sp. | New York | USA | *Thinopyrum intermedium* | 2019 | Field collection | OL342923 | OL314463 |
| NC19004-2 | 4 | *Phyllachora* sp. | New York | USA | *Thinopyrum intermedium* | 2019 | Field collection | | OL314464 |
| NC19004-3 | 4 | *Phyllachora* sp. | New York | USA | *Thinopyrum intermedium* | 2019 | Field collection | OL342924 | OL314465 |
| NC19026-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Triticale* | 2019 | Field collection | OL342917 | OL314466 |
| NC19026-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Triticale* | 2019 | Field collection | OL342918 | OL314467 |
| NC19026-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Triticale* | 2019 | Field collection | OL342919 | |
| NC19029-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | OL342925 | OL314468 |
| NC19029-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | OL342926 | OL314469 |
| NC19029-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | | OL314470 |
| NC19030-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | OL342927 | OL314471 |
| NC19030-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | OL342928 | OL314472 |
| NC19030-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Unknown* | 2019 | Field collection | OL342929 | OL314473 |
| NC19032-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Fall panicum* | 2019 | Field collection | OL342930 | OL314474 |
| NC19032-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Fall panicum* | 2019 | Field collection | OL342931 | OL314475 |
| NC19032-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Fall panicum* | 2019 | Field collection | OL342932 | OL314476 |
| NC19034-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Fescue* | 2019 | Field collection | OL342933 | OL314477 |
| NC19034-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Fescue* | 2019 | Field collection | OL342934 | OL314478 |
| NC19034-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Fescue* | 2019 | Field collection | OL342935 | OL314479 |
| NC19035-1 | 4 | *Phyllachora* sp. | Illinois | USA | *Rye* | 2019 | Field collection | OL342936 | OL314480 |
| NC19035-2 | 4 | *Phyllachora* sp. | Illinois | USA | *Rye* | 2019 | Field collection | | OL314481 |
| NC19035-3 | 4 | *Phyllachora* sp. | Illinois | USA | *Rye* | 2019 | Field collection | OL342937 | OL314482 |
| NC19111-1 | 4 | *Phyllachora* sp. | South Dakota | USA | *Brome grass* | 2019 | Field collection | OL342938 | OL314483 |
| NC19111-2 | 4 | *Phyllachora* sp. | South Dakota | USA | *Brome grass* | 2019 | Field collection | OL342939 | OL314484 |
| NC19111-3 | 4 | *Phyllachora* sp. | South Dakota | USA | *Brome grass* | 2019 | Field collection | OL342940 | OL314485 |
| NC19027-1 | 5 | *Phyllachora* sp. | Illinois | USA | *Triticale* | 2019 | Field collection | OL342941 | OL314486 |
| NC19027-3 | 5 | *Phyllachora* sp. | Illinois | USA | *Triticale* | 2019 | Field collection | OL342942 | OL314487 |

**TABLE 1** (Continued)

| Sample ID | Genetic cluster[a] | Species[b] | State | Country | Host | Year collected | Source | GenBank accessions ITS | GenBank accessions LSU |
|---|---|---|---|---|---|---|---|---|---|
| NC19028-1 | 5 | Phyllachora sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342943 | OL314488 |
| NC19028-2 | 5 | Phyllachora sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342944 | OL314489 |
| NC19028-3 | 5 | Phyllachora sp. | Illinois | USA | Unknown | 2019 | Field collection | OL342945 | OL314490 |
| NC19033-2 | 5 | Phyllachora sp. | Illinois | USA | Fall panicum | 2019 | Field collection | OL342946 | OL314491 |
| NC19037-1 | 5 | Phyllachora sp. | Illinois | USA | Rye | 2019 | Field collection | OL342947 | OL314492 |
| NC19037-2 | 5 | Phyllachora sp. | Illinois | USA | Rye | 2019 | Field collection | OL342948 | OL314493 |
| NC19037-3 | 5 | Phyllachora sp. | Illinois | USA | Rye | 2019 | Field collection | OL342949 | OL314494 |

[a]The genetic cluster was determined as a result of the phylogenetic analysis of the combined DNA sequences from the ITS and LSU regions. These are displayed in Figures 2, 3, and Figure S1.

[b]For contemporary material collected from field samples during this study, specimens of Phyllachora from maize were assumed to be P. maydis and specimens from grass species were treated as unknown Phyllachora sp. For herbarium specimens, we included the species name from the herbarium label.

associated with *Phyllachora* sp. stroma on maize from both herbarium and contemporary specimens representing a span of time from 1904 to 2019 (Table 1). Samples of *Phyllachora* sp. 3 on maize were reported and recovered from herbarium samples throughout the Americas including Bolivia, Colombia, Costa Rica, Cuba, Dominican Republic, Guatemala, Mexico, Nicaragua, Puerto Rico, and Trinidad and Tobago prior to the first report of tar spot on maize in the United States. Importantly, the isosyntype specimen of *P. maydis* (BPI638553) collected in Mexico in 1904 and the *P. maydis* isolate (BPI893226) used in the first report of tar spot in the United States in 2015 are both part of *Phyllachora* sp. 3 and isolates of this species have since been recorded in Illinois, Indiana, Iowa, Michigan, Minnesota, and Wisconsin. This represents the widest geographic range of the maize-infecting *Phyllachora* species in the United States among the samples included in this study. However, isolates of *Phyllachora* sp. 3 were also recovered from another 10 host species including monocots and dicots, with a global distribution including 12 countries across South, Central, and North America and the Caribbean, as well as Germany, India, and the Philippines (Table 1; Figure 3). The herbarium samples associated with each of the 10 host species represented morphologically recognized species of *Phyllachora* including *P. graminis*, *P. heraclei*, *P. junci*, *P. chaetochloae*, *P. diplocarpa*, *P. epicampis*, *P. euphorbiaceae*, *P. rottboelliae*, *P. sylvatica*, and *P. vulgata*.

The other two contemporary maize-infecting species, *Phyllachora* sp. 1 and *Phyllachora* sp. 2, have a more limited observed host and geographic range. Both species were only recovered on maize. *Phyllachora* sp. 1 was only recovered from contemporary maize samples from Indiana and Ohio, whereas *Phyllachora* sp. 2 was found on herbarium specimens from Colombia and Puerto Rico and contemporary specimens from Puerto Rico, Mexico (Guerrero, Oaxaca, Puebla, and Veracruz), and the United States (Florida, Illinois, and Michigan).

The other species recovered from maize was *Phyllachora* sp. 4. However, samples only included herbarium specimens from Guatemala and Venezuela and did not include any contemporary maize specimens. However, *Phyllachora* sp. 4 was commonly found among grasses in the United States that are found in proximity to maize production fields in Illinois, South Dakota, and New York (Table 1). Isolates of *Phyllachora* sp. 4 were recovered from six grass species in four tribes in the United States representing a broad host range across a breadth of genetically diverse grass species. *Phyllachora* sp. 5 was the only species not recovered from maize but was found on many of the same grass species as *Phyllachora* sp. 4, including rye, triticale, and fall panicum (Table 1).

While there was limited *Phyllachora* sequence data in GenBank, we were able to include the ITS sequence of 19 isolates representing six recognized species of *Phyllachora* to determine any relationship between the isolates used in this study and those submitted previously to GenBank (Figure 3). In the case of *Phyllachora* sp. 4, two isolates referred to as *P. graminis*, one from *Hordelymus europaeus* in Germany, and one of unknown origin, as well as isolates of *Phyllachora* on *Elymus kamoji* and *Roegneria* sp. from China grouped together with strong Boostrap support (90%) and posterior
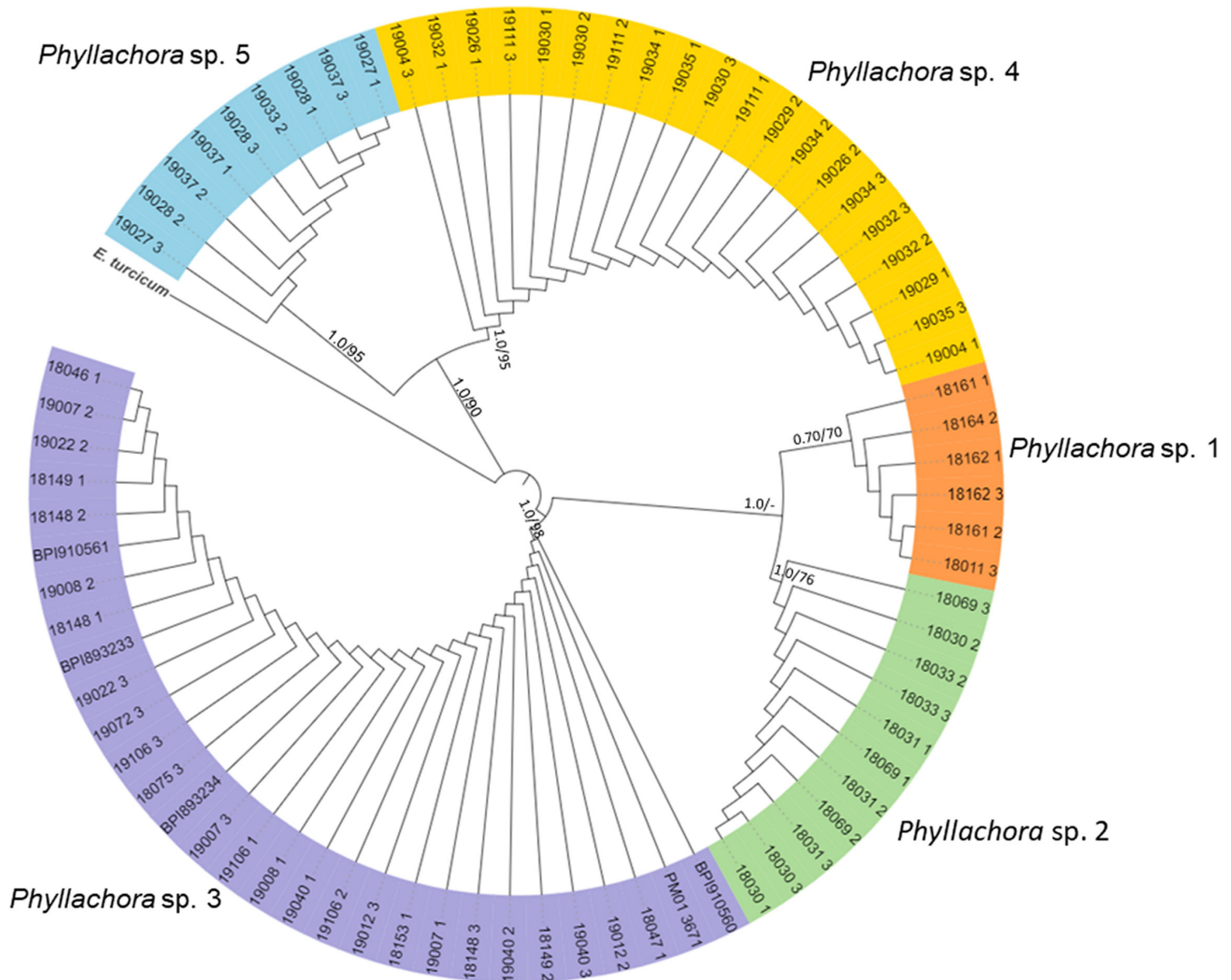
**FIGURE 2** Maximum likelihood phylogenetic tree based on combined ITS and LSU sequence data from the stroma of 76 *Phyllachora* isolates from herbarium and contemporary samples of infected maize and other grass hosts. Values of Bayesian PP > 0.70 or ML BS > 70% are given at nodes at the first and second positions, respectively. *Exserohilum turcicum* CBS 690.71 was used as the outgroup

probability (0.99). There was also an isolate of *P. graminis* from an unknown grass in Canada that grouped together with *Phyllachora* sp. 5, and the herbarium specimen of *P. graminis* from *Agropyron repens* in Germany from this study grouped in *Phyllachora* sp. 3 (Figure 3). Our results support the findings of previous observations that *P. graminis* is a poorly defined polyphyletic species that has often been assigned to tar spot symptom on a variety of grass hosts.

## 4 | DISCUSSION

Since *P. graminis* was described by Persoon in 1785 as *Sphaeria graminis* and then transferred to the genus *Phyllachora* by Fuckel (1870), over 300 species have been recorded on graminaceous hosts, and many more on non-grass hosts. However, Parbery (1967) recognized that there are fewer species associated with grasses and established that there were 95 valid graminicolous *Phyllachora*

species world-wide based on morphological characteristics. In the most complete study of *Phyllachora* species in North America, Orton (1944) identified 45 morphological species from more than 100 host species (Orton, 1944). While this likely represents a significant overestimation of the true number of species in North and Central America, it does demonstrate the vast number of hosts on which *Phyllachora* species have been reported. Our results based on both herbarium and contemporary samples of infected hosts indicate that there are far fewer species of *Phyllachora* in the Americas than indicated by Orton (1944) and Parbery (1967), and the species that are present have a greater host range than previously thought. The predominant species in this study, *Phyllachora* sp. 3, has a broad geographic and host range with the capacity to infect maize throughout South, Central, and North America as well as seven grass species and two dicot species. This phylogenetic species also includes isolates of 11 morphologically determined species of *Phyllachora* (*P. chaetochloae*, *P. diplocarpa*, *P. epicampsis*, *P. euphorbiaceae*, *P. graminis*,

FIGURE 3 Maximum likelihood phylogenetic tree based on ITS sequence data from geographically representative isolates of the five genetic groups of maize and grass-infecting *Phyllachora* from this study and *Phyllachora* species available from GenBank. Values of Bayesian PP > 0.70 or ML BS > 70% are given at nodes at the first and second positions, respectively. Isolates sequenced in this project are denoted in bold. Information on host plant is indicated in blue text and geographic origin in red text
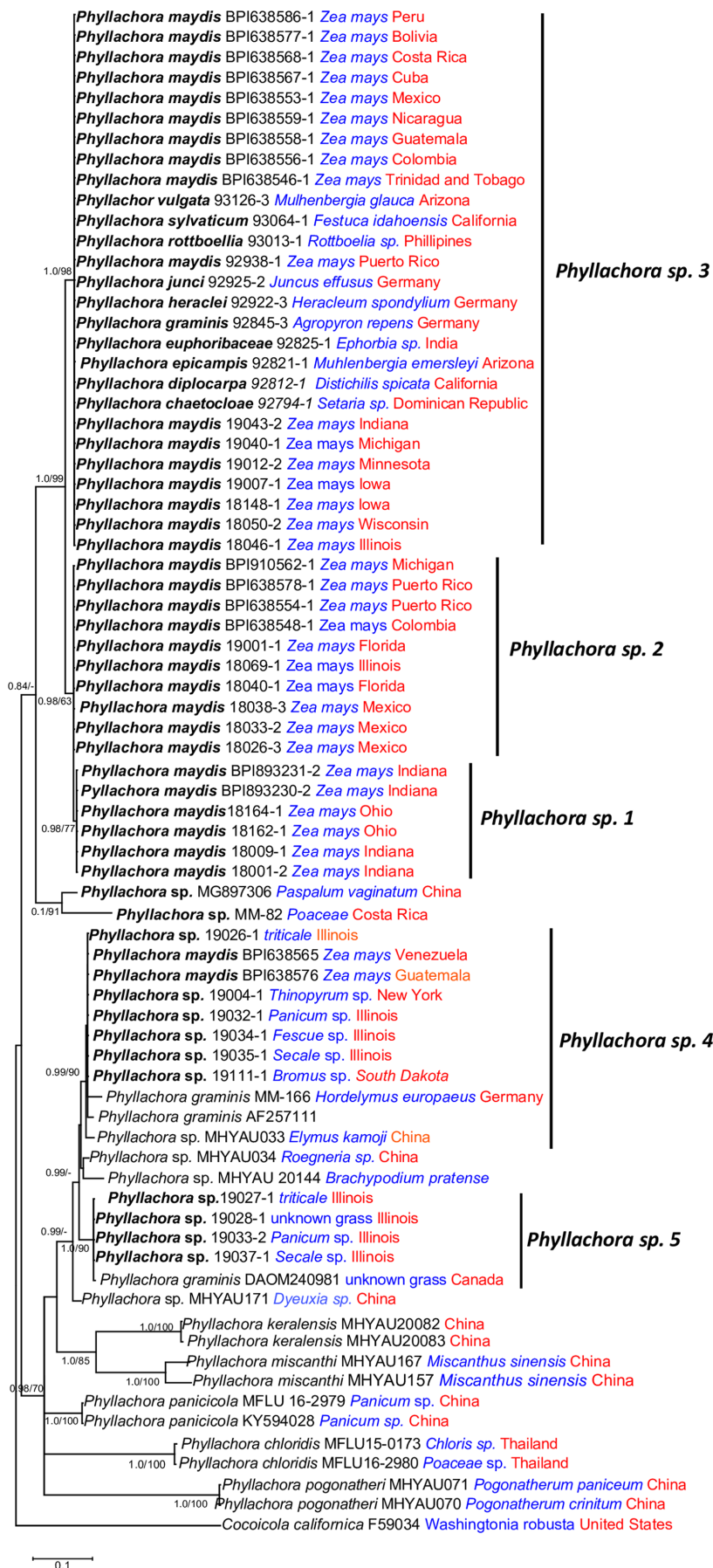
_P. heraclei_, _P. junci_, _P. maydis_, _P. rottboelliae_, _P. sylvatica_, and _P. vulgata_) from herbarium samples collected in the Dominican Republic, Germany, India, Mexico, the Philippines, Trinidad and Tobago, and the United States, indicating global distribution of this species. This expanded host range also now complicates the taxonomic status and the name to be retained by this genetic group. An isolate of _P. graminis_ collected from _Agropyron repens_ from Germany was designated as the lectotype specimen for the genus (Clements & Shear, 1931), and the isolate of _P. graminis_ examined in this study, while not the lectotype was collected from _A. repens_ in Germany, indicating that _P. graminis_ may have precedence for the species name of _Phyllachora_ sp. 3. This would have ramifications for _P. maydis_ as well as several other _Phyllachora_ species in _Phyllachora_ sp. 3 (Table 1; Figure 3) that appear to be synonyms of _P. graminis_. This is based on sequence data from the ITS and/or LSU region and further multi-gene phylogenetic studies of a larger representation of type material from herbaria and contemporary _Phyllachora_ samples from additional hosts is needed for a thorough taxonomic assessment of this genus.

The three maize-infecting species, _Phyllachora_ sp. 1, _Phyllachora_ sp. 2, and _Phyllachora_ sp. 3, have overlapping geographic and host ranges, providing the opportunity for co-infection and genetic exchange. Co-infection on the same leaf tissue by _Phyllachora_ sp. 3 and _Phyllachora_ sp. 1 was observed on four occasions with herbarium samples (BPI893232_1 and BPI893232_2, BPI893231_1 and BPI893231_2, BPI893226_1 and BPI893226_2, BPI893230_1, and BPI893230_2) from three counties in Indiana. A recent fungal community analysis of tar spot lesions on maize found a similar trend with two distinct OTUs occurring on 21 of 22 maize leaf samples from Michigan (McCoy et al., 2019). A similar phenomenon has also been observed in _Albugo candida_, another biotrophic pathogen with a broad host range (McMullan et al., 2015). Races of _A. candida_ were not able to infect a host on their own but were able to co-infect with a race-specific isolate that suppressed host immunity in that host. The offspring of any genetic introgression or recombination resulted in a race with an expanded host range able to infect both plants infected by the parental strains of _A. candida_. A whole-genome comparison of these _A. candida_ races found a mosaic-like genome structure with large portions conserved between races, as well as regions with only 89% sequence similarity. This scenario may explain the wide host range and variation in morphology between hosts in _Phyllachora_ species. Sexual reproduction in _P. maydis_ followed by discharge of infective ascospores commonly occurs on corn leaves annually in maize-producing regions of the United States (Groves et al., 2020; Kleczewski et al., 2019). The presence of multiple maize-infecting species in the mid-western United States, and even on a single infected leaf, combined with frequent sexual recombination, ascospore release and infection, could result in novel populations and/or species of _Phyllachora_ that are more virulent on maize or that have an expanded host range. This may also explain why _Phyllachora_ sp. 3 has such a broad host range, whereas _Phyllachora_ sp. 1 and _Phyllachora_ sp. 2 were only found on maize. Individual populations may gain the ability to infect a new host but are still able to sexually recombine with the rest of the population on the original host species. Given the geographic overlap of many grass species in Central, South, and North America, small populations of _Phyllachora_ sp. 3 may have adapted to infect a novel grass species, while maintaining the ability to recombine with the larger _Phyllachora_ sp. 3 complex, resulting in the expansion of the host range without specialization and speciation.

Speciation has likely occurred in instances where geographic isolation of a new host prevented further introgression with the original population. As maize is commonly grown from Argentina to Canada, it represents a common host for which distinct _Phyllachora_ populations may infect and recombine resulting in potentially new and more virulent populations that are still part of the same species. It is unclear if geographic or genetic barriers lead to speciation between the closely related _Phyllachora_ sp. 1, _Phyllachora_ sp. 2, and _Phyllachora_ sp. 3, but the significant overlap in host and geography would indicate a genetic barrier. While _Phyllachora_ sp. 1 and _Phyllachora_ sp. 2 were only recovered from maize, our sampling scheme was strongly biased toward maize. It is possible that _Phyllachora_ sp. 1 and _Phyllachora_ sp. 2 are present on other grass and non-grass hosts in Central and North America and were not sampled in this study. These non-sampled hosts, if only infected by one of the _Phyllachora_ species, may represent the isolation that led to adaptation and speciation.

For now the name _Phyllachora maydis_ will be retained by _Phyllachora_ sp. 3 as the _P. maydis_ isosyntype material (BPI638553) clustered with this group. However, the presence of three maize-infecting species, the lack of type material of _P. graminis_, and the potential taxonomic synonymy with _P. graminis_ and several other _Phyllachora_ species makes it difficult to determine which of the maize-infecting species will retain the name _P. maydis_. Therefore, we recommend referring to _Phyllachora_ sp. 1, _Phyllachora_ sp. 2, and _Phyllachora_ sp. 3 as the _Phyllachora maydis_ species complex until further morphological and multi-gene phylogenetic studies can properly delineate these species.

In this work, we conducted the most comprehensive assessment of _Phyllachora maydis_ reported to date and provided evidence that our understanding of this species and genera is limited and requires significant attention. The reasons for the emergence of tar spot caused by three species of _Phyllachora_ that have been present in Central America, Mexico, and the Caribbean for over 75 years are still unclear. Several scenarios may explain the recent emergence and severity of tar spot caused by _Phyllachora_ species in the upper Midwest of the United States. While _Phyllachora_ sp. 2 and _Phyllachora_ sp. 3 have been present in both Mexico and Puerto Rico for the last century, it is possible that when the fungus was able to be dispersed via wind and rain to the United States, it could not overwinter in colder climates and the disease could not become established. In fact, according to the herbarium specimens, _Phyllachora_ sp. 3 has been present in the United States since the 1940s in California and Arizona on native grasses but not maize. However, recent studies have demonstrated that _Phyllachora_ spp. can overwinter in Illinois (Kleczewski et al., 2019). Shorter and warmer winters due to climate change could be playing a role in the ability of _Phyllachora_ species to survive further north in the United States. Changes in climate patterns during the growing season may also have an impact on this disease as increased

temperature and precipitation may promote epidemics of this disease. Finally, a change in maize genetics may also play a role in the increased severity of tar spot. Since maize breeding programs were not selecting for resistance to tar spot, any partial resistance that may have been present in U.S. germplasm may have been lost through genetic drift. The loss of this resistance may not have been noticed until *Phyllachora* species arrived in the primary maize-growing region of the United States. The disease remains of minor importance in Mexico and Central American maize production, as resistance to this disease would be selected for in-breeding programs. The most likely scenario for the emergence of tar spot in the United States includes a combination of these factors: (1) introduction of multiple species of *Phyllachora* from Mexico, Puerto Rico, or other Central American countries through movement of infected plant tissue or possible long-distance movement via wind, rain, hurricane/tropical storm system, etc.; (2) change in climate in the Midwestern maize growing region more hospitable to the growth, reproduction, and survival of *Phyllachora* spp.; and (3) lack of resistance in maize germplasm grown in the Midwestern United States.

## CONFLICT OF INTEREST

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## DATA AVAILABILITY STATEMENT

All DNA sequence data generated by this project were deposited in GenBank as accessions OL314402-OL314494 and OL342781-OL342949.

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